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N use efficiency in field vegetable production systems  
—  
Catch crop strategies and fertilization history effects on organic  
fertilizer turnover

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## **Preface**

After reading the title of my thesis you may have wondered whether the world needs another thesis on N use efficiency. There has been substantial progress in many fields concerning N use efficiency of agricultural systems. New fertilizers have been created, new crop varieties have been bred, and new management techniques have been developed. However, due to the world population growth and the associated loss of fertile arable land area, the production intensity on the remaining cultivatable land has increased and will even face further pressure in the future. At the same time, we have to fight global warming and this includes an energy efficient agricultural sector, which does emit as little greenhouse gases as possible. After all, the supply of healthy vegetable products is crucial to our well-being. Unfortunately, the N use efficiency of many agricultural systems, especially the ones involving vegetable production, is still very low, causing direct and indirect environmental as well as financial damage.

Hence, I believe the need to search for agricultural systems, especially in vegetable production, that are more N efficient and thus sustainable has even become more urgent and I hope my thesis can contribute to the progress in this field.

## Table of Contents

<b>1</b>	<b>INTRODUCTION .....</b>	<b>1</b>
1.1	Natural nitrogen cycle .....	1
1.2	Human alteration of the N cycle.....	2
1.3	Nitrogen losses from agricultural systems and vegetable field production.....	2
1.4	Negative consequences of N losses .....	4
1.5	Objective.....	6
1.5.1	Question 1 .....	6
1.5.2	Question 2 .....	8
<b>2</b>	<b>DOES LONG-TERM FARMYARD MANURE FERTILIZATION AFFECT SHORT-TERM NITROGEN MINERALIZATION FROM FARMYARD MANURE? .....</b>	<b>10</b>
2.1	Introduction .....	11
2.2	Materials and methods .....	13
2.2.1	Soil material .....	13
2.2.2	Greenhouse pot experiment.....	13
2.2.3	Analyses .....	14
2.2.4	Balance calculations .....	16
2.2.5	Statistics .....	18
2.3	Results.....	18
2.3.1	Effects of recent fertilization.....	18
2.3.2	Effects of fertilization history .....	19
2.4	Discussion .....	23
<b>3</b>	<b>INFLUENCE OF SOIL FERTILIZATION HISTORY ON DECOMPOSITION OF RECENTLY APPLIED ORGANIC FERTILIZERS .....</b>	<b>28</b>
3.1	Introduction .....	29
3.2	Materials and Methods .....	31
3.2.1	Soil material .....	31

## Table of Contents

3.2.2	Incubation experiment.....	31
3.2.2.1	Recent fertilization treatment .....	31
3.2.2.2	Carbon dioxide .....	32
3.2.2.3	Soil mineral nitrogen and microbial biomass carbon .....	32
3.2.3	Statistics .....	33
3.2.4	Model simulations.....	34
<b>3.3</b>	<b>Results.....</b>	<b>38</b>
3.3.1	Effects of recent fertilization, fertilization history, and their interactions .....	38
3.3.2	Model Simulations .....	41
<b>3.4</b>	<b>Discussion .....</b>	<b>45</b>
3.4.1	Effects of fertilization history on decomposition of recently added fertilizer .....	45
3.4.2	Model simulations.....	47
3.4.3	Conclusions.....	49
<b>4</b>	<b>EFFECT OF WINTER CATCH CROPS ON NITROGEN SURPLUS IN INTENSIVE VEGETABLE CROP ROTATIONS .....</b>	<b>50</b>
<b>4.1</b>	<b>Introduction .....</b>	<b>51</b>
<b>4.2</b>	<b>Materials and Methods .....</b>	<b>53</b>
4.2.1	Experimental sites .....	53
4.2.2	Crop rotation experiments.....	54
4.2.3	Sampling and chemical analyses.....	55
4.2.4	Calculation of N balances .....	57
4.2.5	Statistics .....	58
<b>4.3</b>	<b>Results.....</b>	<b>58</b>
4.3.1	Nitrogen balance .....	58
4.3.2	Catch crop effects on apparent N losses.....	59
4.3.3	Relationship between N input variables and apparent N loss .....	62
<b>4.4</b>	<b>Discussion .....</b>	<b>62</b>
4.4.1	Nitrogen balance and cauliflower crop residues .....	62
4.4.2	Catch crop effects on apparent N loss .....	64
4.4.3	Other factors affecting the N balance .....	66
4.4.4	Conclusions.....	67
<b>5</b>	<b>GENERAL DISCUSSION AND CONCLUSIONS.....</b>	<b>68</b>
<b>5.1</b>	<b>Fertilization history effects on decomposition.....</b>	<b>68</b>
5.1.1	Effects on net N mineralization.....	69

## Table of Contents

---

5.1.2	Effects on gross C and N turnover .....	70
5.1.3	Biodiversity and basic ecosystem functions.....	72
5.1.4	Technical remarks – considered processes and applied methods .....	73
5.1.5	Conclusion .....	74
<b>5.2</b>	<b>Catch crop strategies to reduce N losses .....</b>	<b>75</b>
5.2.1	Effectiveness of catch crops in reducing N balance surpluses and alternative measures .....	75
5.2.2	Technical remarks – the inevitable variation in time and space .....	76
5.2.3	Conclusion .....	77
<b>REFERENCES .....</b>		<b>78</b>

## List of figures

- Figure 2-1: Calculated amounts of N mineralized from soil organic matter ( $\Delta N_{\text{SOM}}$ , Equation 3) and from farmyard manure ( $\Delta N_{\text{FYM}}$ , Equation 5), the amount of N mineralized from farmyard manure that was not immobilized ( $^{\text{available}}\Delta N_{\text{FYM}}$ , Equation 6), and the amount of N that was immobilized by soil microflora ( $N_{\text{immo}}$ ), calculated as the difference between  $\Delta N_{\text{mic}}^{+\text{FYM}}$  and  $\Delta N_{\text{mic}}^{-\text{FYM}}$ . Different letters within a variable indicate significant differences between fertilization history treatments ( $p < 0.05$ ). *Error bars* represent 1 SEM ( $n = 4$ ). ..... 22
- Figure 3-1: Part of the soil organic matter module of the Daisy model (Abrahamsen and Hansen 2000) that was used in this study, modified by introducing soil microbial residual pools ( $\text{SMR}_x$ ). ..... 35
- Figure 3-2: Measured (data points) and simulated (lines) soil mineral N contents (SMN; top row), soil microbial biomass C contents (MBC; second row), accumulated  $\text{CO}_2$ -C release ( $\Delta \text{CO}_2\text{-C}$ ; third row), and added organic matter C (AOM-C; bottom row) in  $\text{g kg}^{-1}$ . Columns represent recent fertilization treatments. Note the different scale of ordinates in the case of MBC and  $\Delta \text{CO}_2\text{-C}$ . For sake of clarity, measured values of  $\Delta \text{CO}_2\text{-C}$  were only presented for the measurement dates that were also available in SMN and MBC. .... 41
- Figure 3-3: Initial values (measurement date 1, RCO) of microbial biomass C (MBC) and soil mineral N (SMN) contents in  $\mu\text{g g}^{-1}$  dry soil. Different letters above the columns indicate significant ( $p < 0.05$ ) differences between fertilization history treatments in MBC (upper case) and in SMN (lower case), respectively. .... 42
- Figure 4-1: Time schedules of all crop rotations. Solid lines: preceding cauliflower crops and succeeding crops, respectively, dashed lines: catch crops. Catch crop treatments: “co” fallow control, “R” winter rye, “F” fodder radish, “S” sudangrass, “B” bunch onion. “x” end of considered crop rotation after establishment of succeeding crop failed in the first experiment at the Großbeeren site. .... 56
- Figure 4-2: Nitrogen inputs of the experimental crop rotations in  $\text{kg ha}^{-1}$ , separated into different components, SMN: soil mineral N in soil depth section 0–90 cm, CR: N in crop residues of preceding cauliflower crop, Total: sum of N in SMN, CR, and mineral

fertilization (not shown). For each component, sample size = 139. Circle: arithmetic mean, hinges: 25% and 75% quantile, whiskers: minimum and maximum values. ....	59
Figure 4-3: Frequency distribution of the CC effect, i.e. the difference in apparent N loss [kg ha <sup>-1</sup> ] of the catch crop treatment minus that of the corresponding control treatment. Data comprises all sites, years, and CC treatments ( $n = 97$ ). .....	60
Figure 4-4: Catch Crop effect, i.e. the differences in apparent N loss in kg ha <sup>-1</sup> of the catch crop treatment minus that of the corresponding control treatment (a) and maximum aboveground N contents of catch crops in kg ha <sup>-1</sup> (b), averaged across all experiments. * Significantly different from zero according to one-sample t-test ( $p < 0.05$ ), catch crops not sharing a common letter (above boxes in 4a) were significantly ( $p < 0.05$ ) different from each other according to a non-parametric post hoc test as suggested by Siegel and Castellan (1988). Sample sizes: sudangrass: 12, onion: 16, rye: 40, radish: 36. Circle: arithmetic mean, hinges: 25% and 75% quantile, whiskers: minimum and maximum values.....	61

## List of Tables

Table 2-1: Plant N uptake ( $N_{\text{PLANT}}$ ), soil mineral N content at the start ( $N_{\text{minSTART}}$ ) and end ( $N_{\text{minEND}}$ ) of the pot experiment, change in microbial N during the pot experiment ( $\Delta N_{\text{mic}} = N_{\text{micEND}} - N_{\text{micSTART}}$ ), microbial N content ( $N_{\text{micEND}}$ ), basal respiration (basal resp <sub>END</sub> ), and metabolic quotient ( $q\text{CO}_2\text{END}$ ) at the end of the experiment (means $\pm$ 1 SEM ( $n = 4$ )).	20
Table 2-2: Two-way ANOVA results ( $p$ values) for the effects of “recent fertilization”, “fertilization history”, and their interaction on plant N uptake ( $N_{\text{PLANT}}$ ), soil mineral N content at the start ( $N_{\text{minSTART}}$ ) and end ( $N_{\text{minEND}}$ ) of the pot experiment, change in microbial N during the pot experiment ( $\Delta N_{\text{mic}} = N_{\text{micEND}} - N_{\text{micSTART}}$ ), microbial N content ( $N_{\text{micEND}}$ ), basal respiration (basal resp <sub>END</sub> ), and metabolic quotient ( $q\text{CO}_2\text{END}$ ) at the end of the experiment.	21
Table 2-3: Soil Ct, soil Nt, soil microbial N ( $N_{\text{mic}}$ ), basal respiration (basal resp), and metabolic quotient ( $q\text{CO}_2$ ) at the start of the pot experiment, before the application of the recent FYM fertilization treatment (means $\pm$ 1 SEM ( $n = 4$ )).	21
Table 2-4: Coefficients of correlation between soil properties and nitrogen mineralization from newly added farmyard manure for the treatments that received a recent FYM fertilization. Mean values ( $n = 12$ ) for the two pot replications were used.	23
Table 3-1: Default Daisy parameters (Hansen 2002) used in all model simulations.	36
Table 3-2: Two-way ANOVA results ( $p$ values) for the effects of fertilization history (H), recent fertilization (R), and their interaction (H x R) on the net change in microbial biomass C contents ( $\Delta\text{MBC}$ ), the net change in soil mineral N contents ( $\Delta\text{SMN}$ ), and accumulated $\text{CO}_2\text{-C}$ evolution ( $\Delta\text{CO}_2\text{-C}$ ).	39
Table 3-3: Weighted residual sum of squares ( $w\text{RSS}$ ) and Akaike’s information criterion ( $AICc$ ) for measured and simulated, accumulated $\text{CO}_2$ release ( $\Delta\text{CO}_2\text{-C}$ ), microbial biomass C contents (MBC), and soil mineral N contents (SMN) as affected by recent fertilization and the consideration of fertilization history effects in the model.	42
Table 3-4: Parameters fitted in the model simulations considering fertilization history effects.	44
Table 4-1: Soil properties and climate conditions at the experimental sites.	54



## List of abbreviations

abbreviation	used unit	meaning
$\alpha$	[-]	level of significance
$\Delta\text{CO}_2\text{-C}$	$\text{g C kg}^{-1}$ soil	accumulated carbon evolution as carbon dioxide gas since start of incubation experiment (Chapter 3)
$\Delta\text{MBC}$	$\text{g C kg}^{-1}$ soil	net change in soil microbial carbon content since start of incubation experiment (Chapter 3)
$\Delta\text{N}_{\text{FYM}}$	$\mu\text{g N g}^{-1}$ soil	nitrogen mineralization from recently applied farmyard manure (Chapter 2)
$\Delta\text{N}_{\text{mic}}$	$\mu\text{g N g}^{-1}$ soil	change in soil microbial biomass nitrogen content between the start and end of pot experiment (Chapter 2)
$\Delta\text{N}_{\text{mic}}^{-\text{FYM}}$	$\mu\text{g N g}^{-1}$ soil	change in soil microbial biomass nitrogen content between the start and end of pot experiment, without recent farmyard manure fertilization (Chapter 2)
$\Delta\text{N}_{\text{mic}}^{+\text{FYM}}$	$\mu\text{g N g}^{-1}$ soil	change in soil microbial biomass nitrogen content between the start and end of pot experiment, with recent farmyard manure fertilization (Chapter 2)
$\Delta\text{N}_{\text{SOM}}$	$\mu\text{g N g}^{-1}$ soil	nitrogen mineralization from soil organic matter (Chapter 2)
$\Delta\text{N}_{\text{SOM}}^{-\text{FYM}}$	$\mu\text{g N g}^{-1}$ soil	nitrogen mineralization from soil organic matter, without recent farmyard manure fertilization (Chapter 2)
$\Delta\text{N}_{\text{SOM}}^{+\text{FYM}}$	$\mu\text{g N g}^{-1}$ soil	nitrogen mineralization from soil organic matter, with recent farmyard manure fertilization (Chapter 2)
$\Delta\text{SMN}$	$\text{g N kg}^{-1}$ soil	net change in soil mineral nitrogen content since start of incubation experiment (Chapter 3)
$\lambda$	[-]	power parameter of Box-Cox power transformation
AIC	[-]	Akaike's information criterion
$AIC_c$	[-]	Akaike's information criterion, corrected for sample size
ANOVA	[-]	analysis of variance
$\text{AOM}_x$	[-]	added organic matter pools, used in model simulations

## List of abbreviations

$\text{available } \Delta N_{\text{FYM}}$	$\mu\text{g N g}^{-1} \text{ soil}$	nitrogen mineralized from recently applied farmyard manure that is not immobilized by the microbial biomass (Chapter 2)
$\text{basal resp}_{\text{START}}$	$\text{ng CO}_2\text{-C g}^{-1} \text{ soil h}^{-1}$	basal respiration of soil microbial biomass at the start of pot experiment (Chapter 2)
$\text{basal resp}_{\text{END}}$	$\text{ng CO}_2\text{-C g}^{-1} \text{ soil h}^{-1}$	basal respiration of soil microbial biomass at the end of pot experiment (Chapter 2)
C	[-]	carbon
$\text{CaCl}_2$	[-]	calcium chloride
CC	[-]	catch crop
$\text{C}_{\text{mic}}$	$\mu\text{g C g}^{-1} \text{ soil}$	soil microbial biomass carbon content (Chapter 2)
$\text{CO}_2$	[-]	carbon dioxide
CR	[-]	crop residues
DON	[-]	dissolved organic nitrogen
EF	$\text{g SMB-C (g C)}^{-1}$	carbon use efficiency of particular soil microbial biomass pool and particular substrate, used in model simulations
$\text{EF}_{\text{tot}}$	$\text{g SMB-C (g C)}^{-1}$	total carbon use efficiency of microbes, used in model simulations
FM	[-]	farmyard manure (Chapter 3)
FYM	[-]	farmyard manure (Chapter 2)
H	[-]	fertilization history treatment
$\text{H}_2$	[-]	molecular hydrogen
HCO	[-]	fertilization history: nil (control) (Chapter 3)
HCR	[-]	fertilization history: crop residues (Chapter 3)
HFM	[-]	fertilization history: farmyard manure (Chapter 3)
HPB	[-]	fertilization history: pine bark (Chapter 3)
HSD	[-]	honestly significant difference
KOH	[-]	potassium hydroxide
MB	[-]	microbial biomass (Chapter 2)
MBC	$\text{g C kg}^{-1} \text{ soil}$	soil microbial biomass carbon content (Chapter 3)
n	[1]	sample size
N	[-]	nitrogen
$\text{N}_2$	[-]	molecular nitrogen

## List of abbreviations

$N_2O$	[-]	nitrous oxide
net $\Delta N$	$\mu g\ N\ g^{-1}\ soil$	net nitrogen mineralization (Chapter 2)
net $\Delta N^{-FYM}$	$\mu g\ N\ g^{-1}\ soil$	net nitrogen mineralization, without recent farmyard manure fertilization (Chapter 2)
net $\Delta N^{+FYM}$	$\mu g\ N\ g^{-1}\ soil$	net nitrogen mineralization, with recent farmyard manure fertilization (Chapter 2)
$NH_3$	[-]	ammonia
$NH_4^+$	[-]	ammonium
$N_{immo}$	$\mu g\ N\ g^{-1}\ soil$	nitrogen immobilization during pot experiment (Chapter 2)
$N_{mic_{START}}$	$\mu g\ N\ g^{-1}\ soil$	soil microbial nitrogen content at the start of pot experiment (Chapter 2)
$N_{mic_{END}}$	$\mu g\ N\ g^{-1}\ soil$	soil microbial nitrogen content at the end of pot experiment (Chapter 2)
$N_{min}$	$\mu g\ N\ g^{-1}\ soil$	soil mineral nitrogen content (Chapter 2)
$N_{min_{START}}$	$\mu g\ N\ g^{-1}\ soil$	soil mineral nitrogen content at the start of pot experiment (Chapter 2)
$N_{min_{END}}$	$\mu g\ N\ g^{-1}\ soil$	soil mineral nitrogen content at the end of pot experiment (Chapter 2)
$NO$	[-]	nitric oxide
$NO_2^-$	[-]	nitrite
$NO_3^-$	[-]	nitrate
$N_{PLANT}$	$mg\ N\ plant^{-1}$	total nitrogen content of plant biomass (Chapter 2)
$N_{plant_{START}}$	$\mu g\ N\ g^{-1}\ soil$	total nitrogen content of plant biomass at the start of pot experiment (Chapter 2)
$N_{plant_{END}}$	$\mu g\ N\ g^{-1}\ soil$	total nitrogen content of plant biomass at the end of pot experiment (Chapter 2)
PB	[-]	pine bark
PE	[-]	priming effect
qCO <sub>2</sub>	$ng\ CO_2-C\ \mu g^{-1}\ C_{mic}\ h^{-1}$	metabolic quotient: basal respiration rate divided by microbial biomass (Chapter 2)
qCO <sub>2</sub> <sub>START</sub>	$ng\ CO_2-C\ \mu g^{-1}\ C_{mic}\ h^{-1}$	metabolic quotient: basal respiration rate divided by microbial biomass, at the start of pot experiment (Chapter 2)
qCO <sub>2</sub> <sub>END</sub>	$ng\ CO_2-C\ \mu g^{-1}\ C_{mic}\ h^{-1}$	metabolic quotient: basal respiration rate divided by microbial

## List of abbreviations

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		biomass, at the end of pot experiment (Chapter 2)
$r$	[-]	Pearson product-moment correlation coefficient
R	[-]	recent fertilization treatment
RCO	[-]	recent fertilization: nil (control) (Chapter 3)
RCR	[-]	recent fertilization: crop residues (Chapter 3)
RFM	[-]	recent fertilization: farmyard manure (Chapter 3)
RPB	[-]	recent fertilization: pine bark (Chapter 3)
SEM	[-]	standard error of the mean
SIR	[-]	substrate induced respiration
SMB <sub>x</sub>	[-]	soil microbial biomass pools, used in model simulations
SMN	g N kg <sup>-1</sup> soil	soil mineral N content (Chapter 3)
SMR <sub>x</sub>	[-]	soil microbial residual pools, used in model simulations
soil Ct	mg C g <sup>-1</sup> soil	soil total carbon content
soil Nt	mg N g <sup>-1</sup> soil	soil total nitrogen content
SOM	[-]	soil organic matter
SOM <sub>x</sub>	[-]	previously existing, non-living soil organic matter pools, used in model simulations
$wRSS$	[-]	weighted residual sum of squares

## Zusammenfassung

In der vorliegenden Studie wurden zwei Fragestellungen bearbeitet, die beide das Ziel verfolgen, die Stickstoff(N)-Ausnutzungseffizienz in landwirtschaftlichen Systemen zu steigern und damit schädliche N-Überschüsse zu vermeiden: 1) Hat die langjährige organische Düngung einen Einfluss auf den Abbau kürzlich applizierter organischer Dünger? Wenn ja, ist dieser Effekt relevant für die N-Düngungspraxis und sollte in Düngungsberatungsprogrammen eingebaut werden? Die Hypothese war, dass relevante Effekte nur bei schwer abbaubaren organischen Düngern auftreten während bei leicht abbaubaren organischen Düngern die Düngungshistorie keine Rolle spielt. 2) Können die hohen N-Bilanzüberschüsse im deutschen, intensiven Freilandgemüsebau durch den Einsatz von Winterzwischenfrüchten (ZF) deutlich reduziert werden? Die Hypothese war, dass durch den Einsatz von ZF die Bilanzüberschüsse der betrachteten zweijährigen Fruchtfolgen um mindestens 30 kg N ha<sup>-1</sup> reduziert werden können.

Die erste Hypothese wurde überprüft, indem der Abbau organischer Dünger in Böden, die sich einzig in Hinblick auf die organische Düngungshistorie unterschieden, gemessen wurde. Dabei wurde ein Topfversuch im Gewächshaus durchgeführt, bei dem kompostierter Stallmist eingesetzt wurde, sowie ein Inkubationsversuch im Labor, bei dem Weißkohlmateriale, kompostierter Stallmist und Kiefernrinde als Dünger appliziert wurden. In beiden Versuchen gab es eine Durchkombination von historischer und rezenter Düngung, was die Detektion von Anpassungseffekten der Bodenlebewesen an einen bestimmten organischen Dünger ermöglichte. Im Topfversuch wurde die Brutto-N-Mineralisation des zugeführten Stallmistes mit Hilfe einer N-Bilanz abgeschätzt. Diese N-Bilanz konnte auf Basis der gemessenen Gehalte an mineralischem Boden-N, Pflanzen-Gesamt-N und N in der mikrobiellen Biomasse zum Start und Ende des zehnwöchigen Versuches aufgestellt werden. Im fünfmonatigen Inkubationsversuch wurden zu fünf Terminen die Gehalte an mineralischem Boden-N und mikrobieller Biomasse bestimmt sowie die Freisetzung von Kohlendioxid kontinuierlich gemessen. Die Daten wurden unter anderem dafür genutzt, mit Hilfe eines mechanistischen Simulationsmodells die Bruttoumsatzraten der rezent applizierten Dünger zu schätzen. Die Ergebnisse beider Studien zusammen deuteten darauf hin, dass es Effekte der Düngungshistorie auf den Abbau von Stallmist und Kiefernrinde gab, während es keine

Effekte auf den Abbau von leicht abbaubarem Kohlmateriale gab. Daher wurde die Hypothese in Hinblick auf den Einfluss der Abbaubarkeit des organischen Düngers angenommen. Allerdings ergaben die beobachteten Effekte kein konsistentes Bild in Hinblick auf die Richtung der Effekte auf die Kohlenstoff(C)- und N-Mineralisierung. Des Weiteren waren die Effekte auf die Netto-N-Mineralisation generell sehr klein. Daher kann die Düngungshistorie für die Abschätzung der N-Freisetzung aus frisch zugeführtem organischen Dünger in der landwirtschaftlichen Praxis vernachlässigt werden.

Zur Überprüfung der zweiten Hypothese wurden an drei Standorten in Deutschland, die sich in Hinblick auf Bodeneigenschaften und Klima deutlich unterschieden, Feldversuche durchgeführt. Dafür wurden typische, intensive Gemüsefruchtfolgen mit einem hohen N-Input, einer Blumenkohlkultur im Sommer des ersten Jahres und einer weiteren Gemüsekultur im Sommer des zweiten Jahres angelegt. Um Variabilitäten der Wetterbedingungen zu erfassen, wurden an allen drei Standorten die Versuche in zwei oder drei aufeinander folgenden Jahren auf neuer Fläche wiederholt angelegt. Die Prüffaktoren waren "Termin", d.h. die Kulturperiode des Blumenkohls und der Saatzeitpunkt der ZF (früh, spät), sowie die "ZF", d.h. keine ZF, eine winterharte ZF oder eine abfrierende ZF. Das Versuchsdesign war eine Block-Spaltanlage. Zur Beurteilung der Effektivität der Fruchtfolgestrategien in Bezug auf die Reduzierung der N-Bilanzüberschüsse wurden N-Bilanzen aufgestellt. Diese N-Bilanzen berücksichtigten die Gehalte an mineralischem N im Boden, die Gesamt-N-Mengen in oberirdischen Pflanzenorganen, N in Ernterückständen des Blumenkohls und mineralische N-Düngung. Die Ergebnisse ergaben, dass die mittleren Bilanzüberschüsse der Kontrollvarianten (ohne ZF) bei  $217 \text{ kg N ha}^{-1}$  lagen. Trotz dieser hohen Überschüsse konnten die ZF, gemittelt über alle Standorte und Experimente, die N-Bilanzen nur um  $13 \text{ kg N ha}^{-1}$  reduzieren. Dabei hatten die Prüffaktoren Termin und ZF einen unbedeutenden Einfluss auf die N-Bilanzen. Diese Ergebnisse legen nahe, dass der Einsatz von Winter-ZF in den untersuchten intensiven Gemüsefruchtfolgen nicht immer erfolgreich ist. Die Hypothese, dass ZF in den untersuchten Systemen die N-Bilanzüberschüsse deutlich verringern, konnte nicht bestätigt werden. Darüber hinaus zeigen die Ergebnisse, dass der verlustfreie Transfer der von der ZF aufgenommenen N-Menge an die Folgefrucht ein besonders kritischer Schritt bei dieser Technik ist.

Beide in dieser Studie untersuchten Ansätze, eine verbesserte N-Ausnutzungseffizienz in landwirtschaftlichen Systemen zu erreichen, erwiesen sich in den gewählten Umgebungen als

nicht effektiv. Alternative Maßnahmen könnten hier größeres Potential besitzen. Dabei sind insbesondere Ansätze zu nennen, die die insgesamt eingebrachte N-Menge reduzieren können. Techniken der sensorgestützten, teilschlagbezogenen Landwirtschaft werden dabei eine bedeutende Rolle spielen, da sie durch eine räumlich präzisere Bewässerung und Düngung einen reduzierten N-Input ohne Ertragsverluste erlauben.

## Summary

The current study dealt with two questions that target potential options to increase the nitrogen (N) use efficiency of agricultural systems and hence avoid environmentally harmful N losses: 1) Does long-term organic fertilization affect the decomposition of recently added organic fertilizers? If so, is this effect relevant to N fertilization practice and hence should be included in fertilizer recommendation systems? The hypothesis was that relevant effects only occur for recalcitrant organic fertilizers while for readily decomposable organic fertilizers, the fertilization history does not play a role. 2) Can the high N balance surpluses in German intensive field vegetable production systems be substantially reduced by cultivation of winter catch crops (CC)? The hypothesis was that the N balance surpluses of the investigated two-year crop rotations can be reduced by a significant amount of more than 30 kg N ha<sup>-1</sup>.

The first hypothesis was tested by applying organic fertilizers to soils that only differed in organic fertilization history. A greenhouse pot experiment with composted farmyard manure and a laboratory incubation experiment with cabbage material, composted farmyard manure, and pine bark as organic fertilizers were conducted. In both experiments, historic and recent fertilization were combined so as to enable the detection of possible “adaptation” effects of the soil biota to a certain fertilizer. In the pot experiment, which lasted 10 weeks, gross N mineralization was estimated by using a balance calculation, including contents of plant N, soil mineral N, and microbial biomass N at the start and end of the experiment. In the incubation experiment, contents of soil mineral N and microbial biomass were determined at five dates while carbon dioxide evolution was monitored continuously over the entire incubation period of five months. Data from the incubation experiment were also used to estimate gross turnover rates of recently added fertilizers by adopting a mechanistic modeling approach. The results of both studies combined indicated that fertilization history had effects on the decomposition of farmyard manure and pine bark, not however on the decomposition of readily decomposable cabbage material. Hence, the hypothesis was accepted in that fertilization history effects depended on the type of fertilizer. However, fertilization history effects showed no consistent trend with respect to increase or decrease in carbon (C) and N mineralization and the effects on net N mineralization were minor in magnitude. Hence, in



agricultural practice, fertilization history effects can be neglected for the prediction of N mineralization from recently applied organic fertilizers.

The second hypothesis was tested by performing field experiments at three sites in Germany, which differed in edaphic and climatic conditions. Typical intensive vegetable crop rotations were set up, comprising a high input of mineral N fertilizers, a cauliflower crop at the start of the rotation, and a succeeding vegetable crop in the summer of the second year. The experiments were repeatedly performed in two or three consecutive years on new plots at each site to account for variability in weather conditions. Two experimental factors were arranged in a split-plot design: factor “date”, i.e. the cultivation period of cauliflower crop and subsequent sowing of the CC (early, late), and factor “type of CC”, i.e. no CC (control), a cold-hardy CC, or a non-cold-hardy CC over the winter period. To assess the effectiveness of the different crop rotation strategies, N balances were calculated on the basis of fertilizer inputs and measured contents of aboveground plant N, soil mineral N, and crop residue N. The results suggested that the average N balance surplus was 217 kg N ha<sup>-1</sup> in the control treatments without a CC. In spite of these high N surpluses, the cultivation of CC reduced the N balance surplus, on average across all sites and experiments, by only 13 kg N ha<sup>-1</sup>, when compared to the control treatments. In some cases, CC even increased the N balance surplus. The factors date and type of CC only had minor influences on the N balance. Hence, the hypothesis that CC can substantially reduce the N balance surpluses in these systems could not be confirmed. The findings further indicate that the transfer of N taken up by the CC to the succeeding crop is a critical step when adopting this technique.

Both approaches to reduce high N losses from agricultural systems that were investigated in the current study were ineffective under the chosen conditions. Other measures to reduce the N balance surpluses in these systems may have greater potential. In particular, approaches that decrease the total N input should come into consideration. In this context, sensor-based precision support tools, which allow a spatially more accurate irrigation and fertilization and thus reduced N input without yield loss, will play a key role.

# 1 Introduction

## 1.1 Natural nitrogen cycle

Since nitrogen (N) is a constituent of many essential organic compounds such as amino acids and nucleic acids, it is crucial to all living organisms on earth. Although there is a huge pool of N on the earth surface, only < 2% of it is available to organisms because around 20% is firmly bound in sedimentary rocks while the vast bulk is present as inert diatomic nitrogen ( $N_2$ ) gas in the atmosphere (Galloway 1998). To segregate the forms of N that are principally available to organisms from the inert atmospheric  $N_2$  pool Galloway et al. (2004) introduced the expression “reactive N”, which will also be used here. A few microorganisms of the domains Bacteria and Archaea are capable of converting atmospheric  $N_2$  to reactive, reduced N forms (ammonium:  $NH_4^+$ , ammonia:  $NH_3$ ), which are bio-available, a process called biological N fixation (Marschner 1995). Likewise, some microorganisms can sequentially reduce reactive, oxidized N in form of nitrate ( $NO_3^-$ ) to  $N_2$  via the intermediates nitrite ( $NO_2^-$ ), nitric oxide (NO), and nitrous oxide ( $N_2O$ ; Zumft 1997). Apart from these microbially mediated processes and lightning strikes, which transform  $N_2$  into NO, the cycling of reactive N within the biosphere and pedosphere is more or less independent from the atmospheric  $N_2$  pool (Galloway et al. 2004). Hence, the N dynamics of terrestrial ecosystems are dominated by the cycling of reactive N between organically bound and inorganic forms. Two counteractive processes are responsible for this cycling. Microbial N mineralization, also called ammonification, converts organically bound N into  $NH_4^+$ , which can then be transformed to  $NO_3^-$  by nitrification. The reverse process, i.e. the conversion of inorganic N forms (e.g.  $NH_4^+$  and  $NO_3^-$ ) into organic N forms, can be performed by microbes (N immobilization) or by plants (plant N assimilation). The heterotrophic, microbial interplay of mineralization and immobilization has been labeled mineralization–immobilization turnover (Jansson and Persson 1982). In soil solution, both  $NH_4^+$  and  $NO_3^-$  are readily available to plants and microbes, however, both forms behave very differently in soil. As a cation  $NH_4^+$  is subject to exchange processes with negatively charged surfaces, which prevail in most soils, whereas  $NO_3^-$  as an anion can easily be leached downwards in the soil profile with percolating soil water.

## 1.2 Human alteration of the N cycle

Due to several human activities, primarily fossil fuel combustion, cultivation of crops associated or in symbiosis with N fixing microbes, and the production of synthetic N fertilizers, the availability of reactive N has drastically increased (Vitousek et al. 1997). Galloway et al. (2004) estimated global N fluxes and demonstrated that the anthropogenic creation of reactive N increased from 15 to 156 Tg N yr<sup>-1</sup> while the natural creation slightly decreased from 246 to 233 Tg N yr<sup>-1</sup> between 1860 and the early 1990s. Hence the total creation of reactive N rose from 262 to 389 Tg N yr<sup>-1</sup> and the anthropogenic proportion thereof from 6 to 40% during this time. This increase could primarily be attributed to the invention of the Haber-Bosch process that made possible the fixation of atmospheric N<sub>2</sub> by reduction with molecular hydrogen (H<sub>2</sub>) to ammonia on an industrial scale. According to Galloway et al. (2004), the amount of N transformed by the Haber-Bosch process was 100 Tg N yr<sup>-1</sup> in the early 1990s, of which 86% was used for production of synthetic N fertilizers applied in the agricultural sector. The application of synthetic N fertilizers has greatly intensified food production increasing crop yields per unit land area, which facilitated the rapid population growth during the last 150 years (FAO 2009). Unfortunately, the downside of this excessive application of N fertilizers in agriculture are unwanted losses of reactive N to other ecosystems, causing detrimental effects on the environment.

## 1.3 Nitrogen losses from agricultural systems and vegetable field production

Naturally, most terrestrial ecosystems are N limited (Aber et al. 1989). Upon increase of N availability the biomass production of these systems is enhanced until another factor becomes limiting, a necessary consequence of Liebig's Minimum Law, which states that the productivity of an ecosystem is only limited by the scarcest resource (Sprengel 1828, Liebig 1840). The result is a typical asymptotical response curve of plant growth to increasing supply of a mineral nutrient, which in an agricultural context has long been known since the formulation of Mitscherlich's Effect Law (Mitscherlich 1909). When the N balance of an ecosystem is positive, i.e. more N is put in the system than removed by e.g. harvest of crops, the storage of N in biomass, soil solution and soil organic matter increases until a status of N saturation is reached. Nitrogen saturation indicates that the storage capacity of the system has been exceeded so that surplus N is lost via different pathways (Aber et al. 1989). In

agricultural fields, the situation is different from (semi-)natural ecosystems in that these ecosystems comprise only few, if not only one, plant species. Moreover, cultivated fields are subject to management practices like soil tillage, application of pesticides, and excessive fertilization. One consequence of this intensive cultivation is that N fluxes are usually much higher in magnitude and more dynamic than those of natural systems. Hence, high N losses from the soil-plant system to other ecosystems can occur in a short time frame, independent of the system's long-term storage capacity for N. Vegetable field production systems are especially prone to high N losses for following reasons:

1. Many field vegetables are sold as a fresh product so that visible loss in quality, e.g., due to suboptimal N supply, immediately leads to loss in marketability of the product (Wismer 2009, p. 28).
2. Field vegetable products are usually harvested during the developmental stage of the crop when the N demand of the plant is very high and thus soil mineral N contents need to be kept at a high level. This is accounted for in fertilizer recommendation systems, e.g., in Feller et al. (2010), which suggest to keep a minimum soil mineral N content of 40 kg ha<sup>-1</sup> until harvest of e.g. cauliflower (*Brassica oleracea* L. var. *botrytis* L.) and broccoli (*Brassica oleracea* L. var. *italica* Plenck).
3. Many vegetable crops, especially *Brassica* species like cauliflower and Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* (DC.) Zenker) have a very low harvest index, so that a large proportion of the biomass and hence N content is left on the field as crop residues after harvest (Feller et al. 2010). These plant residues are readily decomposed in the soil and often contain both easily available carbon (C) and N, potentially promoting N leaching and gaseous N losses by denitrification.
4. A range of vegetable crops like lettuce are very shallow-rooted (maximum rooting depth < 30 cm) so that soil mineral N in deeper soil layers can easily be leached during the growing period after heavy rain and irrigation events.
5. Many vegetable crops whose harvested product is located belowground, i.e. tuber and root crops, are preferably grown on sandy soils in order to produce a good product quality and for convenience of harvesting and cleaning of the product (Simonne and Hochmuth 2003), e.g. carrot (Wonneberger and Keller 2004, p. 122) and asparagus (Wonneberger and Keller 2004, p. 319). Since the hydraulic conductivity of sandy soils is very high and hence their

water holding capacity is very low, the risk of leaching in these soils is high (Kurunc et al. 2011).

6. For most vegetable crops that are cultivated in temperate climate zones, intensive irrigation is necessary during the summer period to guarantee a high product quality. This further increases the risk of N leaching, especially for crops cultivated on sandy soils (see point 5 of this list).
7. Vegetable production, compared to agricultural production of e.g. cereals, is very cost and labor intensive, so that fertilizer costs are only a very small part of total costs. For example, according to cost and return studies performed by the University of California for various crops produced in California, the proportion of fertilizer materials and application costs of total production costs constituted only 4% in cauliflower (UCCE 2004a) and 3% in lettuce (UCCE 2004b) but 14% in grain wheat (UCCE 2004c) and 25% in field corn (UCCE 2004d).
8. The harvest date of vegetable crops is dictated by the continuous demand for fresh products since most products can only be kept fresh for up to a few weeks (Ezeike and Hung 2009, Table 19.1). Hence, a flexible harvest date for purpose of reducing N losses is no option.

#### **1.4 Negative consequences of N losses**

The main pathways of N losses from the soil-plant system are leaching of nitrate and dissolved organic N (DON) and gaseous losses of N oxides and ammonia (Vitousek et al. 1997). These losses have direct and indirect negative impacts on other ecosystems, the most relevant of which are summarized in the following list.

1. Leaching of N to surface waters can cause eutrophication of these systems greatly disturbing the community structure of organisms (Smith et al 1999).
2. Nitrate in drinking water can cause methemoglobinemia in infants and an association between negative developmental effects in offspring and the maternal ingestion of nitrate is under debate (Fan and Steinberg 1996).
3. Nitrogen oxides are secondary greenhouse gases (Forster et al. 2007), contribute to “summer smog”, and form acid rain (Crutzen 1979).

4. Nitrous oxide is a potent greenhouse gas with a global warming potential of 298, relative to an equivalent mass of carbon dioxide (CO<sub>2</sub>) and a time frame of 100 years (Forster et al. 2007). In addition, nitrous oxide leads to the formation of NO and NO<sub>2</sub> in the stratosphere, which contribute to the depletion of the ozone layer (Crutzen 1970).
5. Ammonia volatilization and subsequent deposition in natural ecosystems can cause soil acidification and N saturation (Krupa 2003).

Since reactive N compounds can be converted into one another by many different processes as described in section 1.1, reactive N lost from the soil-plant system can lead to direct negative effects or, after conversion to other N forms, to indirect negative effects on the environment. In this way, a single molecule of reactive N can negatively impact the environment several times, an effect that has been termed the “nitrogen cascade” (Galloway et al. 2003). In non-agricultural ecosystems, the long-term input of reactive N by elevated atmospheric N deposition causes a slow conversion from N limited to N saturated conditions in decades (Aber et al. 1989). The magnitude of N deposition is typically in the range between 0 and 50 kg N ha<sup>-1</sup> yr<sup>-1</sup> and its increase can mainly be attributed to increased losses of N from agriculture (Galloway et al. 2004). In the long term, this N enrichment strongly affects these ecosystems by reducing species richness (Vitousek et al. 1997), which impairs the stability of these systems as demonstrated by increased temporal and spatial variability in basic ecosystem functions like respiration (McGrady-Steed et al. 1997, Naeem and Li 1997) and increased susceptibility to environmental perturbations and exotic species invasions (Hooper et al. 2005).

One should also keep in mind that the production, transport, and application of N fertilizers is accompanied by a high energy demand (FEES and Helikson 1991), which additionally contributes to environmental pollution, foremost by emission of greenhouse gases and other air pollutants.

Apart from the above-mentioned environmental consequences and looked from the perspective of a producer, N losses are also unwanted since they increase fertilizer and labor costs as well as cause high variability in crop yields.

To decrease these unwanted N losses and hence improve the nitrogen use efficiency of vegetable field production systems was the motivation for this study.

## 1.5 Objective

The bulk of N losses from the soil-plant system in vegetable fields is derived from the inorganic N pool of the soil. This is because inorganic N in form of  $\text{NH}_4^+$  is quickly nitrified to  $\text{NO}_3^-$ , which is the primary source of N losses by leaching and denitrification (Subbarao et al. 2006). Hence, in order to take effective measures to reduce N losses, the regulation of soil inorganic N availability is the most promising approach. Soil inorganic N availability in agricultural systems is determined by numerous factors, many of which can be manipulated by farming practice (Di and Cameron 2002). In this respect, the most relevant management options comprise:

1. Type, amount, technique, and timing of N fertilizer application
2. Crop choice and crop rotation
3. Crop residue removal/treatment
4. Soil tillage techniques
5. Scheduling of management events

The objective of the present thesis was to answer two questions, which fall within the scope of point 1 and 2, respectively, of the abovementioned management options.

### 1.5.1 Question 1

Rates of N fertilization are commonly derived from the difference in expected N demand of the crop and expected soil inorganic N availability during the considered period (Feller et al. 2010). When organic N fertilizers are involved, the N release from these fertilizers should ideally be considered in order to obtain a good projection of soil inorganic N availability (Schilling 2000, p. 391 ff.). This projection, however, is a very challenging task due to the complex nature of the decomposition process. Decomposition of organic matter in soil is for the most part microbially mediated and thus controlled by numerous factors that influence the metabolic activity of the microbial community. In this respect, the most important factors are

soil temperature and soil moisture (Cabrera et al. 2005) and the chemical composition of the decomposing matter (Heal et al. 1996). Moreover, it has been demonstrated that long-term organic fertilization can have a lasting effect on a couple of microbial properties, e.g., the microbial community structure (Dambreville et al. 2006) and the activity of microbial enzymes involved in decomposition of organic matter (Carpenter-Boggs et al. 2000). Do these alterations also affect the short-term (weeks–months) decomposition of recently added organic fertilizers? Does the turnover rate of an organic fertilizer increase after decades of application? The few studies that have targeted this issue have not led to a consistent picture (Fauci and Dick 1994, Hadas et al. 1996, Fließbach et al. 2000, Langmeier et al. 2002, Mallory and Griffin 2007). Yet, this question is on the one hand of academic interest and on the other hand could be relevant to fertilizer recommendation systems, depending on the magnitude and duration of potential effects.

**Question:**

**Does long-term organic fertilization with a certain fertilizer lead to increased decomposition rates of this fertilizer after recent application compared to its decomposition in a soil that has received this fertilizer for the first time?**

**Hypothesis:**

**Effects of long-term organic fertilization on short-term decomposition of recently applied fertilizer only occur with recalcitrant organic fertilizers whereas there is no effect with easily decomposable fertilizers since the microbial community structure and activity can adjust quickly enough to compensate for initial differences, at least in a period of months.**

To test this hypothesis, two studies were performed:

**Study 1.** This experiment was conducted in the greenhouse with pot-grown plants and involved farmyard manure as an organic fertilizer applied or not applied to three soils differing in fertilization history (**Chapter 2**)

**Study 2.** A laboratory incubation experiment was conducted under controlled abiotic conditions, which comprised four soils differing in fertilization history and three contrasting organic fertilizers (**Chapter 3**).



### 1.5.2 Question 2

Many vegetable crops leave high amounts of inorganic N in the soil and organically bound N in crop residues after harvest. This N is subsequently at high risk of being lost, especially during the winter period when high leaching rates occur in temperate regions. To prevent N losses, the use of “catch crops” (CC) has been demonstrated to be very effective and workable in many agricultural production systems (e.g. Vos and van der Putten 2004, Beaudoin et al. 2005, Askegaard and Eriksen 2008). Catch crops are intended to take up inorganic N that would otherwise be lost, store it temporarily in biomass, and make it available to the succeeding crop after incorporation of CC residues (Thorup-Kristensen et al. 2003). However, the success of growing CC to prevent N losses depends on many factors and the effects on the N nutrition of the following crop may even be negative (Burket et al. 1997). The key factors determining the CC effects are the amount of N that would be lost without growing a CC, the proportion of this N that can be taken up by the potential CC, and the fraction of N in CC biomass that can be transferred to the succeeding crop after incorporation of CC residues (Thorup-Kristensen 2001, Thorup-Kristensen et al. 2003). For the reasons described in section 1.3, field vegetable production systems are especially prone to high N losses. However, the cultivation of winter CC in these systems is still not commonly used measure to prevent these losses. This may on the one hand be a result of low environmental awareness of responsible people as well as their low acceptance towards alterations in management practices in general. On the other hand, there is still a lack of knowledge about crop rotation strategies (crop choice, cultivation period, time of incorporation etc.) for different field vegetable production systems that can both reduce N losses and guarantee the financial competitiveness of the system. In many vegetable production systems, the cultivation of CC is complicated by the short fallow period between harvest of the last “cash crop” in autumn and planting of the next “cash crop” in early spring. This restricts the choice of CC to crops that can quickly develop a deep root system and have a high uptake capacity for N even at cool temperatures. Furthermore, due to the frequent cultivation of *Brassica* species in many vegetable crop rotations, the use of crucifer CC is not recommended for phytosanitary reasons. Unfortunately, among the crucifer species are some of the most effective CC, e.g. fodder radish (Kristensen and Thorup-Kristensen 2004). Therefore, the identification of suitable crop rotation strategies in typical intensive field vegetable production systems was the aim of this substudy.

**Question:**

**Can N losses be substantially reduced in typical field vegetable production systems by cultivation of winter CC?**

**Hypothesis:**

**The cultivation of winter CC can effectively reduce N losses in typical two-year vegetable crop rotations by an amount of N that is relevant to fertilization practice (i.e. > 30 kg N ha<sup>-1</sup> yr<sup>-1</sup>).**

To test this hypothesis was the objective of study 3:

**Study 3.** At three sites in Germany, differing greatly in soil and climate conditions, field experiments were performed multiple times, testing the effects of CC type ( $n = 3$ ) and sowing date of the CC ( $n = 2$ ) on the N balance of two-year crop rotations (**Chapter 4**).

## **2 Does long-term farmyard manure fertilization affect short-term nitrogen mineralization from farmyard manure?**

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### **Abstract**

One of the challenges in organic farming systems is to match nitrogen (N) mineralization from organic fertilizers and crop demand for N. The mineralization rate of organic N is mainly determined by the chemical composition of the organic matter being decomposed and the activity of the soil microflora. It has been shown that long-term organic fertilization can affect soil microbial biomass (MB), the microbial community structure, and the activity of enzymes involved in the decomposition of organic matter, but whether this has an impact on short-term N mineralization from recently applied organic substances is not yet clear. Here, we sampled soils from a long-term field experiment, which had either not been fertilized, or fertilized with 30 or 60 t ha<sup>-1</sup> year<sup>-1</sup> of farmyard manure (FYM) since 1989. These soil samples were used in a 10-week pot experiment with or without addition of FYM before starting (recent fertilization). At the start and end of this experiment, soil MB, microbial basal respiration, total plant N, and mineral soil N content were measured, and a simplified N balance was calculated. Although the different treatments used in the long-term experiment induced significant differences in soil MB, as well as total soil C and N contents, the total N mineralization from FYM was not significantly affected by soil fertilization history. The amount of N released from FYM and not immobilized by soil microflora was about twice as high in the soil that had been fertilized with 60 t ha<sup>-1</sup> year<sup>-1</sup> of FYM as compared with the non-fertilized soil ( $p < 0.05$ ).

### **Keywords**

decomposition, microbial biomass, organic fertilization, soil adaptation

## 2.1 Introduction

Organic farming systems mainly rely on organic fertilizers such as compost, slurry, and animal and green manure to maintain nitrogen (N) nutrition of the crops. Unlike in most mineral fertilizers, the N contained in organic fertilizers only gradually becomes available after application, through a decomposition process. It is one of the key challenges of organic farming to match N release from organic fertilizers and crop demand for N. However, predicting N release from organic fertilizers is complicated because it is influenced by the physico-chemical properties of the added organic material, abiotic factors like pH, temperature, water and clay content of the soil, and the characteristics of the soil microflora involved in the decomposition process. The characteristics of the soil microflora most relevant for the decomposition process include the soil microbial biomass (MB), the microbial community structure, and the actual microbial activity.

Many incubation studies have demonstrated the relationship between carbon (C) and N mineralization from organic fertilizers and their initial chemical composition. Most authors conclude that the water-soluble C and N contents are good predictors of C and N mineralization during the first weeks of incubation (e.g. Hadas et al. 2004, Jensen et al. 2005). Contents of more recalcitrant components such as cellulose and lignin have been identified as determining C mineralization during later stages (e.g. Trinsoutrot et al. 2000b, Thuries et al. 2002), while long-term (months) N mineralization was often found to be well predicted by total N contents and C/N ratios (e.g. Trinsoutrot et al. 2000a) or the amount of N extractable by a neutral detergent (e.g. Jensen et al. 2005).

The soil microflora decomposes organic matter to obtain nutrients or to use parts of it in redox-reactions delivering chemically bound energy to the microorganisms. Soil MB, the community structure, activity, and physiological properties of the soil microflora, such as nutrient use efficiency or cell nutrient concentration, change in response to the availability of organic matter, which in turn has an effect on the decomposition process. Numerous studies have shown that long-term organic fertilization, as compared with long-term mineral fertilization, can increase contents (per mass of soil) of soil organic matter (SOM) and soil MB (e.g. Houot and Chaussod 1995, Salinas-Garcia et al. 1997, Gunapala and Scow 1998, Kandeler et al. 1999), enhance the activities of enzymes involved in the decomposition process (e.g. Dick et al. 1988, Carpenter-Boggs et al. 2000), and change the microbial community structure (e.g. Dambreville et al. 2006, Ruppel et al. 2007, Stark et al. 2008). Also, incubation experiments indicate that the easily degradable proportion of SOM, and thus

the content of potentially mineralizable N is increased by long-term organic fertilization (Hadas et al. 1996, Whalen et al. 2001).

Hence, long-term organic fertilization can alter SOM contents, soil MB, soil enzyme activities, and the microbial community structure. It is not clear however, whether this results in long-term fertilization leading to modified decomposition rates of newly added organic matter. This open question is both of academic interest and of practical relevance in fertilizer recommendation systems. Most simulation models predicting N mineralization from recently added organic matter assume that the decomposition process can be described by first-order kinetics, with a rate constant depending only on the chemical properties of the material and abiotic factors, such as soil temperature and soil moisture (e.g. Molina et al. 1983, Abrahamsen and Hansen 2000). The effects of fertilization history on properties of the soil microflora are not considered. The rationale behind this is that the MB, microbial community structure and microbial activity of soil adjust quickly to newly added organic matter, so that initial properties of the soil microflora can be ignored in the prediction of short-term N mineralization from organic fertilizer. Most published studies on this issue support this theory (e.g. Fauci and Dick 1994, Hadas et al. 1996, Langmeier et al. 2002, Stark et al. 2008), although others have found an effect of long-term fertilization history on C mineralization (Fließbach et al. 2000) or N mineralization (Mallory and Griffin 2007) from newly added organic matter.

Many previous studies compared treatments that comprised a combination of different management activities, such as plant protection, tillage operations, cultivated crops, and fertilizer use (e.g. Gunapala and Scow 1998, Breland and Eltun 1999, Sanchez et al. 2001, Burger and Jackson 2003, Oehl et al. 2004, Stark et al. 2008). However, very few studies compared treatments that differed only in fertilizer inputs. The aim of the present study was to test the hypothesis that long-term organic fertilization has a stimulating effect on short-term N mineralization from recently added organic fertilizer. We compared treatments that only differed in fertilization history and calculated net N mineralization from recently added fertilizer on the basis of measured net changes in different N pools.

## 2.2 Materials and methods

### 2.2.1 Soil material

The soil was taken from a long-term field experiment, which was established on the experimental site in Großbeeren (52°20'N, 13°19'E) in 1989. The soil was a loamy sand (Arenic Luvisol) with 810 mg g<sup>-1</sup> sand, 140 mg g<sup>-1</sup> silt, and 50 mg g<sup>-1</sup> clay. In the plow layer (0–20 cm), the content of organic C was 6–12 mg g<sup>-1</sup>, the content of total N 0.5–1.0 mg g<sup>-1</sup>, the soil pH was 6.5, and the soil bulk density was 1.6 g cm<sup>-3</sup> (Rühlmann, personal communication). Three treatments of this field experiment were used in the present experiment: (1) no fertilization since 1989, (2) 30 t ha<sup>-1</sup> year<sup>-1</sup> of fresh farmyard manure (FYM) since 1989, and (3) 60 t ha<sup>-1</sup> year<sup>-1</sup> of FYM since 1989 (corresponding to 6 and 12 t ha<sup>-1</sup> year<sup>-1</sup> of dry FYM, respectively). These treatments will be referred to as “fertilization history”. Manure, originated from a dairy cow farm, was applied once a year in autumn or spring during the fallow period. An exclusively horticultural crop rotation with one or two vegetable crops per year was maintained. For the last 5 years (2003–2007), the crops were celery, broccoli/endive, mangold, artichoke, and leek. All crop residues were removed from the field after harvest. The soil samples were taken as composite samples ( $n = 6$ ) from the plow layers of each of the four replicate plots in late February 2008. Keeping the samples from the four field replicates separate generated 12 samples (three fertilization histories  $\times$  four field replicates), which were stored for 14 days at 8°C in the dark before processing. In the long-term field experiment, soil total C concentrations (soil Ct) and soil total N concentrations (soil Nt) were annually determined using a CNS-Analyzer VARIO EL (Elementar Hanau). Since the plow layer of the soil was carbonate-free, soil Ct values can be considered as concentrations of organic C.

### 2.2.2 Greenhouse pot experiment

To investigate the mineralization of N from recently added FYM, a greenhouse pot experiment was performed. For this purpose, each of the 12 field-moist soil samples taken from the long-term field experiment was mixed thoroughly and split into two equivalent samples. One sample was fertilized with 7.6 g of lyophilized and ground (2 mm) manure per kilogram of dry soil. These treatments will be referred to as “recent fertilization”. The recent fertilization rate corresponded to 60 t ha<sup>-1</sup> of fresh FYM assuming a soil bulk density of 1.6 g cm<sup>-3</sup> and a plow layer depth of 20 cm. Per unit of dry matter, the applied FYM contained

362 mg g<sup>-1</sup> total C, 30.4 mg g<sup>-1</sup> total N, and 1.3 mg g<sup>-1</sup> mineral N (NO<sub>3</sub><sup>-</sup>-N + NH<sub>4</sub><sup>+</sup>-N). The applied FYM originated from the same cow farm as the FYM applied in the field experiment, which on average for the years 1989 to 2007 contained 363 mg g<sup>-1</sup> total C and 24.7 mg g<sup>-1</sup> total N. Immediately after mixing, equivalents of 1.1 kg of dry soil were fitted in 1.3 L pots in two replicate sets. A density of approximately 1.1 g cm<sup>-3</sup> was achieved by compacting the soil in the pots to the same height. Each pot was planted with a fodder radish (*Raphanus sativus* ssp. *oleiformes*) seedling, which had newly developed two true leaves and on average, based on a sub-sample of 68 seedlings, contained 1.24 mg N. Fodder radish has a high capacity for N uptake and was thus used to remove mineral N from the pot so as to prevent N losses by denitrification or leaching and to transform the N into a form that can be readily analyzed at the end of the experiment. In the greenhouse, all pots (3 fertilization histories x 4 field replicates x 2 recent fertilization treatments x 2 pot replicates = 48) were arranged in a randomized block design, blocked by pot replicate. All pots were frequently weighed and gravimetric water content adjusted to 160 mg g<sup>-1</sup> dry soil (approximately 70% of water holding capacity). No drainage water from the pots was observed. The duration of the experiment was 70 days from planting on March 14 to harvest on May 23 in 2008. During this period, the average temperature in the greenhouse was 18.6°C (min, 11.1°C and max, 30.1°C), and the average daily irradiance of photosynthetic active radiation in the greenhouse was 87 W m<sup>-2</sup> (min, 16 W m<sup>-2</sup> and max, 193 W m<sup>-2</sup>).

### 2.2.3 Analyses

To determine soil mineral N and soil microbial biomass content, duplicate soil samples were taken at the start and end of the greenhouse pot experiment. The samples from the start were taken after mixing and sieving to 2 mm, but before splitting the samples ( $n = 12$ ) for the fertilization treatment described above (recent fertilization). At the end of the greenhouse pot experiment, the complete soil material of each pot ( $n = 48$ ) was homogenized after removing the fodder radish roots.

For contents of soil mineral N ( $N_{min} = \text{NO}_3^- \text{-N} + \text{NH}_4^+ \text{-N}$ ), the soil samples were kept at -20°C for not more than 5 days prior to analysis. Then a dry mass equivalent of 25 g of moist soil was extracted with 100 ml of 0.0125 M CaCl<sub>2</sub> solution and  $N_{min}$  concentration was determined colorimetrically in the extract using an EPOS analyzer, Eppendorf, Germany (Bassler and Hoffmann 1997).

To determine soil microbial biomass-C content ( $C_{mic}$ ), the substrate induced respiration (SIR) activity was measured at 22°C with an automatic infrared gas analyser (Heinemeyer et

al. 1989) and the regression equation found by Anderson and Domsch (1978) was used to convert maximum initial respiration rate to  $C_{mic}$ . To measure SIR activity, the gravimetric water content was adjusted to  $120 \text{ mg g}^{-1}$  dry soil (approximately 50% of water holding capacity) and the soil was mixed with glucose at a rate of 3 mg of glucose per gram of moist soil. The samples from the start of the greenhouse pot experiment had been air dried at the start of the experiment and were rewetted and incubated for 2 weeks at  $25^{\circ}\text{C}$  prior to SIR analysis. The samples from the end of the experiment were stored at  $4^{\circ}\text{C}$  immediately after harvest for a few days prior to SIR analysis. In addition to SIR activity, basal respiration was measured in the same way, but without adding glucose, and the metabolic quotient ( $q\text{CO}_2$ ) was calculated as the ratio of basal respiration divided by  $C_{mic}$ . The metabolic quotient reflects changes in substrate and energy use efficiency and can thus indicate changes in the microbial community structure (Anderson 1994). Determinations of SIR activity and basal respiration took place within one month after the end of the pot experiment. Total plant (shoot+root) dry matter ( $n = 48$ ) was measured at the end of the pot experiment (May 23) after carefully removing soil residues from the roots. Total carbon and nitrogen in the overall plant material (shoot and root material pooled) was determined using a CNSAnalyzer VARIO EL (Elementar Hanau).



### 2.2.4 Balance calculations

The simplified mass balance for N was calculated considering five different pools: plant N, soil mineral N, microbial biomass N, soil organic matter N (not including the MB), and farmyard manure N. Following components were used.

$N_{\text{PLANT}} = N_{\text{plant}_{\text{END}}} - N_{\text{plant}_{\text{START}}}$	Plant N uptake, calculated as the difference between total plant N content at the end and start of the pot experiment. For $N_{\text{plant}_{\text{START}}}$ , the average N content of the fodder radish seedling was used.
$N_{\text{min}_{\text{START}}}, N_{\text{min}_{\text{END}}}$	Soil mineral N content at the start and end of the pot experiment. For the treatments that received recent FYM fertilization, $N_{\text{min}_{\text{START}}}$ included the amount of mineral N added with FYM ( $0.0013 \text{ g N g}^{-1} \text{ dry FYM}$ ).
$\Delta N_{\text{mic}} = N_{\text{mic}_{\text{END}}} - N_{\text{mic}_{\text{START}}}$	Net N immobilization by soil microflora, calculated as the difference of total microbial biomass-N content between the end and start of the pot experiment, assuming a $C_{\text{mic}}/N_{\text{mic}}$ ratio of 10 (e.g. Chapin et al. 2002)
$\text{net}\Delta N$	Apparent net N mineralization (Equation 1).
$\Delta N_{\text{SOM}}$	N mineralization from soil organic matter (microbial biomass not included) (Equation 3)
$\Delta N_{\text{FYM}}$	N mineralization from farmyard manure (Equation 5)
$^{\text{available}}\Delta N_{\text{FYM}}$	N mineralized from farmyard manure that was not immobilized by soil microflora (Equation 6)

The components  $N_{\text{PLANT}}$ ,  $N_{\text{min}_{\text{START}}}$ ,  $N_{\text{min}_{\text{END}}}$ , and  $\Delta N_{\text{mic}}$  were measured or derived from measurements whereas  $\text{net}\Delta N$ ,  $\Delta N_{\text{SOM}}$ ,  $\Delta N_{\text{FYM}}$ , and  $^{\text{available}}\Delta N_{\text{FYM}}$  were calculated as follows. Where necessary, superscript indices ( $^{-\text{FYM}} / ^{+\text{FYM}}$ ) were used to refer to the treatments with and without recent fertilization with FYM, respectively.

In the first step,  $\text{net}\Delta N$  was calculated for every pot according to Equation 1, assuming that the plants took up only mineral N.

$$\text{net}\Delta\text{N} = \text{N}_{\text{PLANT}} + \text{N}_{\text{min}_{\text{END}}} - \text{N}_{\text{min}_{\text{START}}} \quad (1)$$

For the treatments that did not receive a recent fertilization with FYM, it was assumed that  $\text{net}\Delta\text{N}^{-\text{FYM}}$  consisted of  $\Delta\text{N}_{\text{SOM}}^{-\text{FYM}}$  and  $\Delta\text{N}_{\text{mic}}^{-\text{FYM}}$  (Equation 2).

$$\text{net}\Delta\text{N}^{-\text{FYM}} = \Delta\text{N}_{\text{SOM}}^{-\text{FYM}} - \Delta\text{N}_{\text{mic}}^{-\text{FYM}} \quad (2)$$

Hence,  $\Delta\text{N}_{\text{SOM}}^{-\text{FYM}}$  could then be calculated according to Equation 3.

$$\Delta\text{N}_{\text{SOM}}^{-\text{FYM}} = \text{net}\Delta\text{N}^{-\text{FYM}} + \Delta\text{N}_{\text{mic}}^{-\text{FYM}} \quad (3)$$

For the treatments that received a recent FYM fertilization,  $\text{net}\Delta\text{N}^{+\text{FYM}}$  included the amount of N mineralized from FYM ( $\Delta\text{N}_{\text{FYM}}$ ), N mineralized from SOM ( $\Delta\text{N}_{\text{SOM}}^{-\text{FYM}}$ ) and N immobilized by soil microflora ( $\Delta\text{N}_{\text{mic}}^{+\text{FYM}}$ ) (Equation 4).

$$\text{net}\Delta\text{N}^{+\text{FYM}} = \Delta\text{N}_{\text{FYM}} + \Delta\text{N}_{\text{SOM}}^{-\text{FYM}} - \Delta\text{N}_{\text{mic}}^{+\text{FYM}} \quad (4)$$

Here,  $\Delta\text{N}_{\text{SOM}}^{-\text{FYM}}$  was inserted from Equation 3. Hence, a possible effect of recent FYM fertilization on N mineralization from SOM (priming effect (PE)) was neglected as it was beyond the scope of this study to determine a possible PE by means of isotope techniques. A study by Blagodatskaya and Kuzyakov (2008) suggests that the PE concerning respired C can be expected to be zero or negative when the ratio of added substrate C to microbial C in the soil is higher than 2. In the present study, this ratio ranged between 27 and 38.

Equation 4 was then rearranged to give  $\Delta\text{N}_{\text{FYM}}$  (Equation 5).

$$\Delta\text{N}_{\text{FYM}} = \text{net}\Delta\text{N}^{+\text{FYM}} - \Delta\text{N}_{\text{SOM}}^{-\text{FYM}} + \Delta\text{N}_{\text{mic}}^{+\text{FYM}} \quad (5)$$

The amount of  $\Delta\text{N}_{\text{FYM}}$  that was not immobilized by soil microflora and thus became available to the plants was calculated according to Equation 6.

$$\text{available}\Delta\text{N}_{\text{FYM}} = \Delta\text{N}_{\text{FYM}} - (\Delta\text{N}_{\text{mic}}^{+\text{FYM}} - \Delta\text{N}_{\text{mic}}^{-\text{FYM}}) \quad (6)$$

Here, the total immobilization by soil microflora was considered, calculated as the difference between  $\Delta N_{mic}^{+FYM}$  and  $\Delta N_{mic}^{-FYM}$ .

### 2.2.5 Statistics

All statistical analyses were performed using Statistica 6.1. (StatSoft, Inc, Tulsa, USA). For analysis of variance, the assumption of normal distribution of within-group errors was tested by the Kolmogorov–Smirnov test, while homoscedasticity was checked using the Levene’s test. When the preconditions were met, the type VI sums of squares (effective hypothesis) ANOVA was used in combination with the Tukey’s HSD multiple comparison procedure. When the assumptions were violated, a Kruskal-Wallis test was used instead. Since the block factor of the replicate pots had no significant effect on any of the investigated variables, it was omitted and statistics were done using mean values for the replicate pots. The level of significance  $\alpha$  was set to 0.05 in all cases. Unless stated otherwise, results are presented as mean  $\pm$  1 standard error of the mean (SEM).

## 2.3 Results

### 2.3.1 Effects of recent fertilization

Recent fertilization significantly increased plant N uptake ( $N_{PLANT}$ ) as well as soil mineral N at the start and end of the pot experiment ( $N_{minSTART}$ ,  $N_{minEND}$ ; Tables 2-1 and 2-2). Also, the change in microbial N contents between the start and end of the pot experiment ( $\Delta N_{mic}$ ) was significantly affected by recent fertilization (Table 2-2). For the treatments that received the recent fertilization, net immobilization by the soil microflora occurred whereas without recent fertilization net mineralization was observed (Table 2-1). Consequently, the microbial N content at the end of the experiment ( $N_{micEND}$ ) was higher for the recently fertilized treatments (Tables 2-1 and 2-2). Basal respiration at the end of the pot experiment ( $basal\ resp_{END}$ ) also increased significantly due to the recent fertilization whereas the metabolic quotient ( $qCO2_{END}$ ) was not significantly affected (Tables 2-1 and 2-2).

### 2.3.2 Effects of fertilization history

Fertilization history significantly affected soil Ct, soil Nt, and Nmic<sub>START</sub> (Table 2-3) as well as Nmin<sub>START</sub> and plant N uptake (N<sub>PLANT</sub>) (Table 2-1 and 2-2). All of these properties increased consistently with increasing fertilization in the field history ( $0 < 30 < 60 \text{ t FYM ha}^{-1} \text{ yr}^{-1}$ ; Table 2-1 and 2-3). For the amount of mineral N measured at the end of the pot experiment (Nmin<sub>END</sub>), fertilization history only had a significant effect for the treatments that received a recent fertilization, whereas there was no effect for the treatments without a recent fertilization (Table 2-1), i.e. there was a significant interaction between the two main factors for this variable (Table 2-2).

Table 2-1: Plant N uptake ( $N_{\text{PLANT}}$ ), soil mineral N content at the start ( $N_{\text{minSTART}}$ ) and end ( $N_{\text{minEND}}$ ) of the pot experiment, change in microbial N during the pot experiment ( $\Delta N_{\text{mic}} = N_{\text{micEND}} - N_{\text{micSTART}}$ ), microbial N content ( $N_{\text{micEND}}$ ), basal respiration ( $\text{basal resp}_{\text{END}}$ ), and metabolic quotient ( $q\text{CO2}_{\text{END}}$ ) at the end of the experiment (means  $\pm$  1 SEM ( $n = 4$ )).

R	H	N <sub>PLANT</sub>		N <sub>min</sub> <sub>START</sub> <sup>a</sup>		N <sub>min</sub> <sub>END</sub>		ΔN <sub>mic</sub>		N <sub>mic</sub> <sub>END</sub>		basal resp <sub>END</sub>		qCO <sub>2</sub> <sub>END</sub>	
(t FYM ha <sup>-1</sup> yr <sup>-1</sup> )		(mg N plant <sup>-1</sup> )		————— (μg N g <sup>-1</sup> soil) —————						(ng CO <sub>2</sub> -C g <sup>-1</sup> soil h <sup>-1</sup> )		(ng CO <sub>2</sub> -C μg <sup>-1</sup> Cmic h <sup>-1</sup> )			
0	0	8.3 ± 1.2	a	0.4 ± 0.4	a	0.0 ± 0.0	a	-0.5 ± 0.5	a	6.3 ± 0.6	a	169.8 ± 59.3	a	3.26 ± 0.95	a
0	30	11.9 ± 0.9	b	3.3 ± 0.2	b	0.0 ± 0.0	a	-1.3 ± 0.9	a	8.0 ± 0.9	a	221.1 ± 15.6	a	2.88 ± 0.30	a
0	60	14.0 ± 0.7	c	4.0 ± 0.5	b	0.5 ± 0.2	a	-1.9 ± 1.0	a	8.6 ± 0.6	a	120.7 ± 26.9	a	1.73 ± 0.35	a
60	0	23.1 ± 1.0	A	10.4 ± 0.4	A	1.0 ± 0.4	A	4.6 ± 0.6	A	11.4 ± 0.5	A	269.2 ± 34.7	A	2.79 ± 0.40	A
60	30	28.0 ± 1.1	B	13.2 ± 0.2	B	2.2 ± 0.4	A,B	4.2 ± 1.3	A	13.5 ± 1.3	A	233.8 ± 13.3	A	1.83 ± 0.16	A
60	60	32.7 ± 1.9	C	13.9 ± 0.5	B	3.2 ± 0.4	B	1.6 ± 1.0	A	12.0 ± 0.8	A	214.4 ± 34.1	A	1.83 ± 0.25	A

Different letters after values within a column indicate significant differences between history treatments ( $p < 0.05$ ), separately for the treatments with (uppercase letters) and without (lowercase letters) recent fertilization.

R Recent fertilization

H Fertilization history

<sup>a</sup> Includes mineral N added with FYM for treatments with recent fertilization

The change in microbial N contents between the start and end of the pot experiment ( $\Delta N_{mic}$ ) as well as microbial biomass N ( $N_{mic_{END}}$ ), basal respiration ( $basal\ resp_{END}$ ), and metabolic quotient ( $qCO2_{END}$ ) at the end of the incubation were not significantly affected by fertilization history (Table 2-1 and 2-2).

Table 2-2: Two-way ANOVA results ( $p$  values) for the effects of “recent fertilization”, “fertilization history”, and their interaction on plant N uptake ( $N_{PLANT}$ ), soil mineral N content at the start ( $N_{min_{START}}$ ) and end ( $N_{min_{END}}$ ) of the pot experiment, change in microbial N during the pot experiment ( $\Delta N_{mic} = N_{mic_{END}} - N_{mic_{START}}$ ), microbial N content ( $N_{mic_{END}}$ ), basal respiration ( $basal\ resp_{END}$ ), and metabolic quotient ( $qCO2_{END}$ ) at the end of the experiment.

	$p$ value						
	$N_{PLANT}$	$N_{min_{START}}^a$	$N_{min_{END}}$	$\Delta N_{mic}$	$N_{mic_{END}}$	$basal\ resp_{END}$	$qCO2_{END}$
Recent Fertilization (R)	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0245</b>	0.2395
Fertilization History (H)	<b>0.0000</b>	<b>0.0000</b>	<b>0.0005</b>	0.0781	0.0885	0.1926	0.0546
R x H	0.2713	1.0000	<b>0.0163</b>	0.5006	0.4379	0.3858	0.4985

<sup>a</sup> Includes mineral N added with FYM for treatments with recent fertilization

$p$  values < 0.05 are shown in bold.

Table 2-3: Soil Ct, soil Nt, soil microbial N ( $N_{mic}$ ), basal respiration ( $basal\ resp$ ), and metabolic quotient ( $qCO2$ ) at the start of the pot experiment, before the application of the recent FYM fertilization treatment (means  $\pm$  1 SEM ( $n = 4$ )).

Fertilization History	Soil Ct <sup>a</sup>	Soil Nt	$N_{mic_{START}}$	$basal\ resp_{START}$	$qCO2_{START}$
(t FYM ha <sup>-1</sup> yr <sup>-1</sup> )	(mg C g <sup>-1</sup> soil)	(mg N g <sup>-1</sup> soil)	( $\mu$ g N g <sup>-1</sup> soil)	(ng CO <sub>2</sub> -C g <sup>-1</sup> soil h <sup>-1</sup> )	(ng CO <sub>2</sub> -C $\mu$ g <sup>-1</sup> Cmic h <sup>-1</sup> )
0	6.1 $\pm$ 0.4 a	0.51 $\pm$ 0.03 a	6.8 $\pm$ 0.4 a	140.2 $\pm$ 30.7 a	1.96 $\pm$ 0.43 a
30	7.7 $\pm$ 0.6 ab	0.65 $\pm$ 0.06 a	9.2 $\pm$ 0.5 b	141.6 $\pm$ 27.0 a	1.57 $\pm$ 0.28 a
60	10.1 $\pm$ 0.2 b	0.85 $\pm$ 0.01 b	10.5 $\pm$ 0.5 b	189.4 $\pm$ 18.2 a	1.80 $\pm$ 0.12 a

Different letters after values within a column indicate significant differences between history treatments ( $p < 0.05$ ).

<sup>a</sup> Significant differences according to Kruskal-Wallis multiple comparisons test ( $p < 0.05$ )

The N mineralization from SOM during the pot experiment as calculated from the treatments without the recent fertilization ( $\Delta N_{SOM}$ , Equation 3) as well as the total N mineralization from the recently added FYM ( $\Delta N_{FYM}$ , Equation 5), were not significantly affected by fertilization history (Fig. 2-1). Also, the immobilization of N by soil microflora calculated as the difference between  $\Delta N_{mic}^{+FYM}$  and  $\Delta N_{mic}^{-FYM}$  was not significantly affected by fertilization

history (Fig. 2-1). However, the amount of N mineralized from recently added FYM that was not immobilized and thus became plant available, was significantly higher in the treatments that had been subject to high fertilization rates in the past ( $60 \text{ t of FYM ha}^{-1} \text{ yr}^{-1}$ ), compared to the treatment with no fertilization ( $0 \text{ t of FYM ha}^{-1} \text{ yr}^{-1}$ ; Fig. 2-1).

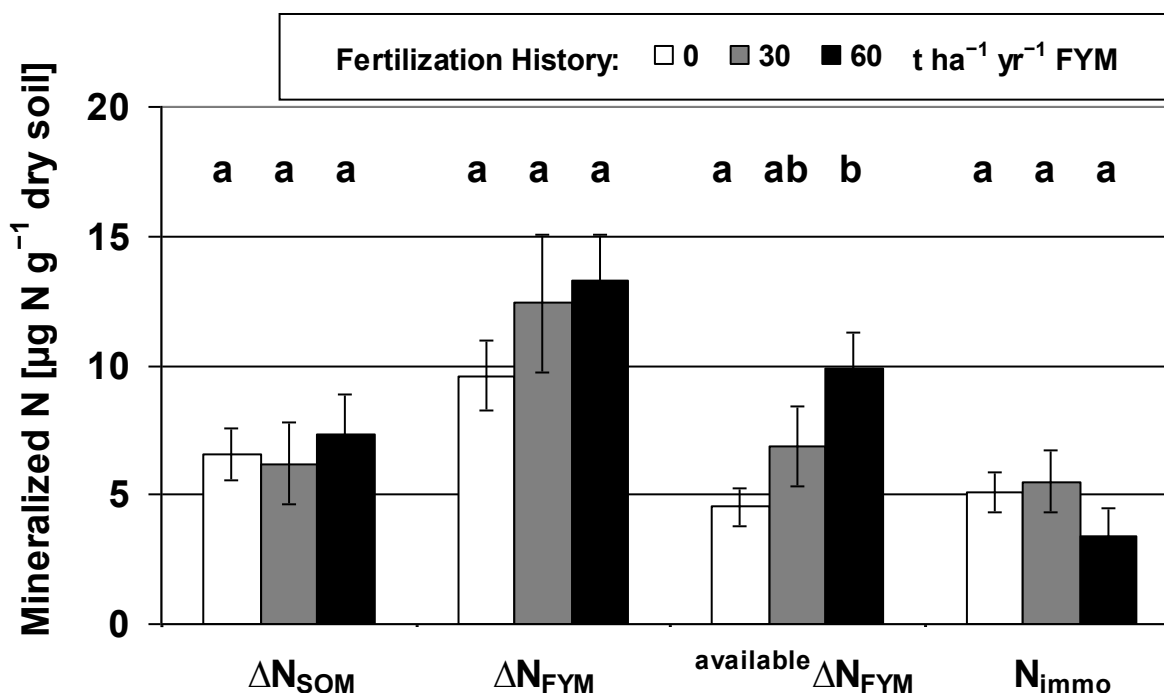


Figure 2-1: Calculated amounts of N mineralized from soil organic matter ( $\Delta N_{\text{SOM}}$ , Equation 3) and from farmyard manure ( $\Delta N_{\text{FYM}}$ , Equation 5), the amount of N mineralized from farmyard manure that was not immobilized ( $^{\text{available}}\Delta N_{\text{FYM}}$ , Equation 6), and the amount of N that was immobilized by soil microflora ( $N_{\text{immo}}$ ), calculated as the difference between  $\Delta N_{\text{mic}}^{+\text{FYM}}$  and  $\Delta N_{\text{mic}}^{-\text{FYM}}$ . Different letters within a variable indicate significant differences between fertilization history treatments ( $p < 0.05$ ). Error bars represent 1 SEM ( $n = 4$ ).

There was no significant correlation between  $^{\text{available}}\Delta N_{\text{FYM}}$  and  $N_{\text{mic}_{\text{START}}}$  but there were significant correlations between  $^{\text{available}}\Delta N_{\text{FYM}}$  and soil Ct, soil Nt, and  $N_{\text{min}_{\text{START}}}$  (Table 2-4), these latter soil properties being strongly inter-correlated (not shown).

Table 2-4: Coefficients of correlation between soil properties and nitrogen mineralization from newly added farmyard manure for the treatments that received a recent FYM fertilization. Mean values ( $n = 12$ ) for the two pot replications were used.

	$\Delta N_{\text{FYM}}$	$\text{available } \Delta N_{\text{FYM}}$
Soil Ct	0.47	<b>0.68</b>
Soil Nt	0.45	<b>0.67</b>
$N_{\text{minSTART}}$	0.40	<b>0.66</b>
$N_{\text{micSTART}}$	0.20	0.41

Significant ( $p < 0.05$ ) values are shown in bold.

## 2.4 Discussion

The results showed that contrasting fertilization treatments since 1989 had induced considerable differences in soil Ct, soil Nt, and  $N_{\text{micSTART}}$  (Table 2-3). In addition, soil mineral nitrogen contents at the start of the pot experiment ( $N_{\text{minSTART}}$ ; Table 2-1) were significantly increased with increasing fertilization rates in the field. This was probably due to different N mineralization rates caused by different SOM contents in the long-term experiment, from which the soil samples were taken (Rühlmann and Geyer 1993). The calculated N mineralization from SOM during the incubation, however, was not significantly different between soil histories ( $\Delta N_{\text{SOM}}$ , Fig. 2-1), probably due to the short time period covered in the pot experiment. Yet, in comparison to  $N_{\text{PLANT}}$  (Table 2-1), which varied between 8.3 and 32.7 mg N pot<sup>-1</sup> as well as  $\Delta N_{\text{FYM}}$  (Fig. 2-1), ranging from 10.6 to 14.6 mg N pot<sup>-1</sup>,  $\Delta N_{\text{SOM}}$  was indeed a relevant N balance component, ranging from 7.2 to 8.1 mg N pot<sup>-1</sup>.

Both main factors of the study – fertilization history and recent fertilization – significantly affected several properties of soils, plants, and microorganisms. However, there were no significant interactions between the main factors regarding  $N_{\text{micEND}}$ , basal resp<sub>END</sub>, qCO<sub>2END</sub>,  $N_{\text{PLANT}}$ , and  $\Delta N_{\text{mic}}$  (Table 2-2), so that changes in these properties due to the recent fertilization were not significantly affected by fertilization history. In addition, the total N mineralization of the recently added FYM was not significantly affected by fertilization history ( $\Delta N_{\text{FYM}}$ , Fig. 2-1).

These results are supported by Fauci and Dick (1994), who reported that soils that had received beef manure or pea vine in the past did not respond differently to recent additions of these supplements than soils that had not received the respective supplement before, with



respect to microbial C and N contents, soil enzyme activity, and respiration. Here, N mineralization was not measured.

In contrast, Mallory and Griffin (2007) reported a fertilization history effect. They applied farmyard manure to soils that had either been mainly fertilized with organic materials or mainly been fertilized with inorganic materials in the past. They reported that the proportion of N released as nitrate from recently added manure at the end of a 282-day incubation period was significantly lower in the organically fertilized soil. The authors attributed this effect to more active soil microflora that immobilized more N from manure than the microflora in the minerally fertilized soil. These authors further argued that one possible reason why their results did not concur with those of Hadas et al. (1996), Sanchez et al. (2001), and Langmeier et al. (2002) was that the pair of contrasting soils they used was far more disparate than in the other studies. After 11 years of contrasting amendment history they found soil Ct 67% higher and soil Nt 79% higher in the organically fertilized soil than in the minerally fertilized soil. Notably, the soils in the present study did show considerable differences due to the different fertilization histories. Compared to the non-fertilized treatment, the fertilization rate of 60 t ha<sup>-1</sup> yr<sup>-1</sup> FYM resulted in a significant increase in soil Ct, soil Nt, and Nmic, corresponding to 65%, 68%, and 54%, respectively (Table 2-3), but had no significant effect on  $\Delta N_{\text{FYM}}$  (Fig. 2-1).

$\Delta N_{\text{FYM}}$  estimates the total N mineralization from the recently added FYM while <sup>available</sup> $\Delta N_{\text{FYM}}$  gives the mineralized N that became plant available, i.e. that was not immobilized by soil microflora. It should be noted that <sup>available</sup> $\Delta N_{\text{FYM}}$ , which is similar to the N availability parameter measured by Mallory and Griffin (2007), was significantly affected by field history. However, the availability of N was higher in the historically fertilized soil, contradictory to the results of Mallory and Griffin (2007). Correspondingly, the observed changes in Nmic during the pot experiment indicated that N immobilization was lower in the soil that received high organic fertilization in the past as compared to non-fertilized soil and soil that received lower amounts of FYM in the past, although this difference was not significant (Fig. 2-1). Yet, the immobilized N corresponded to 53, 45, and 26% of  $\Delta N_{\text{FYM}}$  for the fertilization history treatments 0, 30, and 60 t FYM ha<sup>-1</sup> yr<sup>-1</sup>, respectively. The results suggest that the consistent trend observed in  $N_{\text{PLANT}}$ , which increased with the rate of FYM fertilization in soil history was a combined result of the differences in  $N_{\text{minSTART}}$ ,  $\Delta N_{\text{SOM}}$ ,  $\Delta N_{\text{mic}}$ , and, in case of recent fertilization, also of  $\Delta N_{\text{FYM}}$ . The significant effect of fertilization history on <sup>available</sup> $\Delta N_{\text{FYM}}$  was

thus in part generated by a non-significant increase in  $\Delta N_{\text{FYM}}$  and in part by a non-significant decrease in N immobilization with increasing rate of FYM fertilization in soil history.

It should be noted that differences in plant growth and associated root exudation might have had an influence on microbial activity and thus FYM decomposition. However, with the methods used, no conclusions can be drawn concerning this potential feedback effect.

In contrast to the results of the present study, Fließbach et al. (2000) reported that after recent addition of straw a higher proportion of straw C was both mineralized and assimilated by the soil microflora during 177 days of incubation in soils that had been managed ‘bio-dynamically’ compared to soils that had been managed ‘conventionally’. The authors attributed this to a higher soil MB content as well as more efficient substrate use by the microorganisms in the ‘bio-dynamic’ treatment. However, N mineralization was not measured in this study.

Hadas et al. (1996) and Langmeier et al. (2002) found no increase in net N mineralization from recently added organic material in soils that were organically fertilized in the past as compared to non-fertilized or minerally fertilized soils despite higher soil MB contents in the organically fertilized soils. This contradicts the effect of fertilization history on  $^{\text{available}}\Delta N_{\text{FYM}}$  found in the current experiment but it is supported by Stark et al. (2008), who reported that the investigated soils – although having distinct microbial community structures due to different fertilization histories – reacted comparably to recent addition of organic material in terms of soil MB contents, enzyme activities, and net N mineralization.

We did not investigate the microbial community structure but even after 19 years of contrasting treatments, the metabolic quotient measured both at the start and the end of the experiment (Table 2-1 and 2-3), was not significantly affected by field history. This suggests that no relevant shift in the energetic efficiency occurred due to long-term organic fertilization. Hence, it is unlikely that the community structures differed greatly between the different soil fertilization history treatments (Anderson 1994, Ruppel et al. 2007), either before or after the recent application of FYM.

Perhaps the differences found in  $^{\text{available}}\Delta N_{\text{FYM}}$  were caused by other factors than initial differences in properties of the soil microflora. The variables  $N_{\text{micSTART}}$  and  $^{\text{available}}\Delta N_{\text{FYM}}$  were significantly affected by fertilization history and were higher in the 60 t FYM ha<sup>-1</sup> yr<sup>-1</sup> treatment compared to the non-fertilized samples. But this does not necessarily mean that the higher microbial biomass had caused the higher N availability from the recently added FYM.

In fact,  $^{available}\Delta N_{FYM}$  was not significantly correlated to  $N_{mic_{START}}$  but there were significant correlations to soil Ct, soil Nt, and  $N_{min_{START}}$  (Table 2-4). Under field conditions, soil Ct and soil Nt might affect N mineralization indirectly, since SOM affects soil temperature and soil moisture, which in turn affect mineralization. These indirect effects, however, were excluded under the controlled conditions of the pot experiment. The results rather suggest that different SOM contents between the fertilization history treatments caused the differences in mineral N availability at the start of the pot experiment ( $N_{min_{START}}$ , Table 2-1). In turn, these differences had an impact on the decomposing activity of the MB, which was limited by N availability. It needs to be mentioned that net N transformation rates as determined in this study can only to a limited extent explain the processes that occurred. Gross nutrient turnover rates determined by means of isotope tracing techniques may contribute to a better understanding of N transformation processes.

Finally, it should be considered that during the 10-week pot experiment on average only 3.2% of the organic N applied in FYM (244.4 mg N) was mineralized and became available ( $^{available}\Delta N_{FYM}$ ) and the observed fertilization history effect corresponded to an absolute increase in  $^{available}\Delta N_{FYM}$  of 2.4% of the added organic N. In comparison, Mallory and Griffin (2007) found between 7% and 27% of the total N added with manure in the soil nitrate pool after 40 weeks of incubation and Hadas et al. (1996) recovered approximately 26% of the total added manure N, corresponding to 10% of organic N added, after 33 weeks of incubation. A longer experimental duration might have also produced a significant effect of fertilization history on  $\Delta N_{FYM}$  in the current experiment.

In summary, the results confirm that long-term fertilization with organic matter in the field has profound effects on a range of chemical and biological soil properties. These changes can also have an effect on the fate of N from recently applied organic matter. This effect of fertilization history on soil N transformations after a recent application of organic matter may be related to different processes, partly investigated in the present experiment. The presented data, together with other published evidence, however, suggest that effects of fertilization history on transformation of recently applied organic matter are not always dominant. Therefore it may not be necessary to include such long-term soil memory effects in fertilizer recommendation systems for horticulture and agriculture, at least for short-term crops.

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### **3      Influence of soil fertilization history on decomposition of recently applied organic fertilizers**

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#### **Abstract**

Long-term organic fertilization, compared to mineral or no fertilization, can change soil organic matter content, microbial biomass content, the microbial community structure, and the activity of enzymes involved in organic matter decomposition. However, it is not clear whether long-term organic fertilization by means of these changes, leads to modified decomposition rates of newly added fertilizers. Therefore, this study aimed to test the hypothesis that fertilization history has an influence only on the decomposition of recalcitrant organic fertilizers and not on less recalcitrant organic materials. Soils used for experimentation were taken from a field experiment where contrasting organic fertilization regimes of: farmyard manure, pine bark, vegetable crop residues, and no fertilization had been applied for 35 years. In a full factorial, laboratory-based incubation experiment, each soil was treated with each of the above-mentioned fertilizers and net C and N mineralization and microbial biomass C contents were monitored over a 147-day period. Collected data was then used to estimate turnover rates of newly added fertilizers with a modeling approach based on the soil organic matter module of the Daisy model. The modeling results suggested that the turnover of farmyard manure and pine bark should be simulated in consideration of a fertilization history effect whereas this was not the case for cabbage material. Hence, the hypothesis that effects of fertilization history on turnover rates of recently added organic fertilizer depend on the type of fertilizer was confirmed. However, the results of the ANOVA indicated that fertilization history had an insignificant effect on net C and N mineralization from recently applied fertilizer and can thus be neglected for fertilization practice.

#### **Keywords**

decomposition, microbial biomass, organic fertilization, soil adaptation, fertilization history effect

### 3.1 Introduction

Predicting nitrogen (N) mineralization from organic fertilizers is necessary to match crop N demand and N availability, especially in organic farming systems, which mainly rely on organic fertilization. However, since decomposition is a multi-level process influenced by numerous factors, the prediction of N availability to plants is often inaccurate. Soil microorganisms play a key role in the turnover of organic matter, which they decompose to obtain mineral nutrients and energy. Apart from abiotic factors, such as soil temperature, soil water content, and soil aeration, the properties of the organic fertilizer itself affect the decomposition process, partly via their impact on soil microflora. Relationships between the chemical composition of organic fertilizers and their carbon (C) and N mineralization have been frequently observed (e.g. Trinsoutrot et al. 2000a, Hadas et al. 2004, Jensen et al. 2005). Aside from the immediate effects of organic fertilization on the soil microflora, various studies have demonstrated that long-term organic fertilization, compared to no or solely mineral fertilization, can increase the microbial biomass content in soil (Houot and Chaussod 1995, Salinas-Garcia et al. 1997, Gunapala and Scow 1998, Kandeler et al. 1999). Also, the microbial community structure can change due to long-term organic fertilization (e.g. Dambreville et al. 2006, Ruppel et al. 2007, Stark et al. 2008) and the activities of enzymes involved in decomposition processes can be enhanced (e.g. Dick et al. 1988, Carpenter-Boggs et al. 2000). However, it is not clear whether long-term fertilization results in the modified decomposition rates of newly added organic matter.

Microorganisms can adapt quickly to new organic substrates by adjusting their metabolic activity, as demonstrated by shifts in substrate utilization patterns and community fatty acid methyl ester profiles within days after addition of different C sources (e.g. Schutter and Dick 2001). From such observations, it could be assumed that within months, the initial differences in the properties of the soil microflora would have a negligible effect on the mineralization of C and N from recently added material. This assumption has been included in most mechanistic simulation models that describe the decomposition process of organic matter by first-order kinetics, with rate constants depending only on the chemical properties of the added material and several environmental factors, as in the Daisy (Abrahamsen and Hansen 2000) and NCSOIL (Molina et al. 1983) models.

While many studies confirm the concept that fertilization history effects on the decomposition of recently applied fertilizer can be neglected (e.g. Fauci and Dick 1994, Hadas et al. 1996, Langmeier et al. 2002, Stark et al. 2008), some do not. For example, Fließbach et al. (2000) reported higher C mineralization from recently added straw in “biodynamically” managed soil

compared to conventionally managed soil (no, mineral, or mineral+organic fertilization). Alternatively, Mallory and Griffin (2007) found that long-term organic fertilization decreased the availability of soil mineral N (SMN) from recently added manure compared to long-term mineral fertilization. In contrast, results from Nett et al. (2010) indicated that net N mineralization from recently added manure was increased in soil fertilized with manure for 20 years compared to non-fertilized soil. Yet, the factors that determine whether fertilization history has an effect on the decomposition of recently added fertilizers are not known. Therefore, the objective of this study was to examine whether such fertilization history effects depend on the chemical characteristics of the applied fertilizers.

It was hypothesized that recalcitrant organic fertilizers decompose faster in a soil which has received the same fertilizer previously, whereas easily decomposable fertilizers are decomposed with the same rate in all soils, irrespectively of the soils' fertilization history. The rationale behind this hypothesis was that recalcitrant fertilizer may require a specialized microbial community, a phenomenon already observed in organic xenobiotics (e.g. Jablonowski et al. 2010), whereas easily decomposable fertilizers do not. In an incubation experiment, the C and N mineralization of three types of organic fertilizers, differing greatly in their decomposability, were compared after their application to soils taken from the site of a long-term field experiment, which had received either of the corresponding fertilizers or no fertilizer for 35 years prior.

## 3.2 Materials and Methods

### 3.2.1 Soil material

The soil used in this experiment was taken from the site of a long-term field experiment established in 1973 in Großbeeren, Germany (52°20'N, 13°19'E). A detailed description of the experimental setup can be found in Rühlmann (2006). To give a brief overview, the field experiment involves a 5-year rotation of vegetable crops (white cabbage (*Brassica oleracea* L. var. *capitata* L.), carrot (*Daucus carota* L.), cucumber (*Cucumis sativus* L.), leek (*Allium porrum* L.), and celeriac (*Apium graveolens* L. var. *rapaceum* (Mill.) DC.)) and incorporates different fertilization strategies, each replicated four times. The soil material used in this study was a loamy sand (88% sand, 6% silt, and 6% clay) with a pH value of 6.6, originated from the Ap horizon of an Arenic Hapludalf. The treatments used, referred to as fertilization history treatments (prefix “H”), were: an unfertilized control (HCO), fertilization with composted, solid farmyard manure from a dairy cow farm (HFM), fertilization with pine bark (HPB), and fertilization with crop residues of the previous crop (HCR). The HCR treatment was introduced in 1981, before this time, the fertilizer used was the solid phase of manure. The HCO, HFM, and HPB treatments were applied consistently since 1973. The average amounts (until 2008) of annually applied total C and N were 2104 kg C ha<sup>-1</sup> and 148 kg N ha<sup>-1</sup> (HFM), 2628 kg C ha<sup>-1</sup> and 23 kg N ha<sup>-1</sup> (HPB), and 1101 kg C ha<sup>-1</sup> and 51 kg N ha<sup>-1</sup> (HCR, since 1981). The HCO treatment received only root residues.

In April 2008, before vegetables were planted, six soil samples were taken from the 0–20 cm layer of each replication plot, obtaining 24 samples (4 replication plots x 6 samples) for each of the four treatments (HCO, HFM, HPB, HCR). The samples of each treatment were pooled, homogenized, and sieved (< 2 mm). Total soil C and N contents were determined using a CNS-Analyzer VARIO EL (Elementar, Hanau, Germany). Until required for further experimentation, samples were stored in polyethylene bags at 4°C.

### 3.2.2 Incubation experiment

#### 3.2.2.1 Recent fertilization treatment

Each of the four soil materials were mixed with either: nothing (control), farmyard manure (FM), pine bark (PB), or crop residues (CR) at a rate equivalent to 2 mg of added C per g dry soil. Before application, FM, PB, and CR were freeze-dried and ground (2 mm) to facilitate homogenization. The amendments are hereafter referred to as recent fertilization treatments



(prefix “R”), indicated by RCO, RFM, RPB, and RCR. Thus, 16 treatments were obtained by applying four recent fertilization treatments to each of the four fertilization history treatments. The FM and PB were obtained from the same sources as in the field experiment and the CR used in this experiment were taken from the white cabbage crop of the control plot in 2008. Per unit of dry matter, total N contents were 30.4 (FM), 4.1 (PB), and 14.5 (CR) mg g<sup>-1</sup> and C/N ratios were 12 (FM), 122 (PB), and 28 (CR).

The gravimetric water content of the soil–fertilizer mixtures was adjusted to 50% water holding capacity, which, depending on the soil, corresponded to 0.14 (HCO), 0.17 (HFM, HPB), and 0.15 (HCR) g H<sub>2</sub>O g<sup>-1</sup> dry soil, respectively. In a 147-day (16.06.2008–10.11.2008) laboratory incubation experiment, net CO<sub>2</sub> release and net changes in SMN and microbial biomass carbon (MBC) contents were measured.

### 3.2.2.2 Carbon dioxide

The CO<sub>2</sub> evolution was determined continuously in a non-destructive way, using 5 replications of each treatment. Each of the 80 vessels (250 ml) was filled with a dry mass equivalent of 25 g of the prepared soil–fertilizer mixtures and CO<sub>2</sub> evolution measured in a Respicond IV analyzer (A. Nordgren Innovations AB, Bygdeå, Sweden) at 22°C. Carbon dioxide released by the sample was trapped in KOH solution (0.6 mM; 10 ml) and the electric conductivity of the KOH solution determined automatically every hour for each vessel. The conductivity was then converted into accumulated CO<sub>2</sub> absorbed using the linear range of the calibration curve of conductivity vs. trapped CO<sub>2</sub> (Nordgren 1988). During the incubation, KOH solution was replaced five times for the treatments recently amended with cabbage (at day 3, 11, 28, 64, and 95) and four times for all other treatments (at day 11, 28, 64, and 95). These replacements also ensured sufficient gas exchange in order to avoid a lack of oxygen in the incubation vessels, which, estimated from the maximal accumulated CO<sub>2</sub> absorption, did not fall below 12% by volume. Values were corrected by taking into consideration the average CO<sub>2</sub> absorption of the empty vessels ( $n = 8$ ). Hourly accumulated CO<sub>2</sub> evolution, measured since the start of the incubation experiment, was converted into daily values of accumulated C released ( $\Delta\text{CO}_2\text{-C}$ ).

### 3.2.2.3 Soil mineral nitrogen and microbial biomass carbon

The process of SMN and MBC content determination was destructive and involved removing 3 replications of each treatment at every one of 5 measurement dates: 0 (immediately after application), 3, 9, 78, and 147 days after application of the recent fertilization. Thus, in total 240 incubation vessels were needed for each, SMN and MBC analysis (16 treatments x 3

replications x 5 measurement dates). Each of the 480 vessels (300 ml) was filled with a dry mass equivalent of 25 g of the prepared soil–fertilizer mixtures and incubated in the same room as the vessels for CO<sub>2</sub> evolution measurements at 22°C in the dark. To avoid a lack of oxygen during the incubation, the samples were opened and aerated on the dates corresponding to the KOH solution replacements in the CO<sub>2</sub> measurements. Removed samples were stored at –20°C prior to analysis.

Contents of SMN (NO<sub>3</sub><sup>–</sup>-N plus NH<sub>4</sub><sup>+</sup>-N) were determined colorimetrically after extraction of the 25 g soil sample (dry mass equivalent) with 100 ml of 0.0125 M CaCl<sub>2</sub> solution (Bassler and Hoffmann 1997) using an EPOS analyzer (Eppendorf, Hamburg, Germany). The observed net changes in SMN contents between the start of incubation and the corresponding measurement date were calculated and referred to as ΔSMN. For each soil, the value of the control treatment (RCO) at day 0 was used as the initial value for all recent fertilization treatments, with the addition of the amounts of mineral N introduced with the recent fertilizer application. These were 7.18, 0.08, and 0.49 μg N g<sup>–1</sup> dry soil in RFM, RPB, and RCR, respectively.

To determine MBC contents, the substrate-induced respiration (SIR) activity was measured at 22°C with an automatic infrared gas analyzer (Type 225 MK3, Analytical Development Company, Hoddesdon, England) according to Heinemeyer et al. (1989). The regression equation suggested by Anderson and Domsch (1978) was used to convert the maximum initial respiration rate to MBC after adding dry glucose at a rate of 3 mg of glucose per gram of dry soil to a sample of 25 g (dry mass equivalent). The observed net changes in MBC contents between the start of incubation and the corresponding measurement date were calculated and referred to as ΔMBC. For each soil, the value of the control treatment (RCO) at day 0 was used as the initial value for all recent fertilization treatments.

### 3.2.3 Statistics

Statistical analyses were performed using R statistics (v. 2.11.1; R Development Core Team 2010). For analysis of variance, the assumption of normal distribution of within-group errors was tested by the Kolmogorov–Smirnov test, while homoscedasticity was checked using the Levene’s test. When assumptions were violated, data was transformed using the Box-Cox power transformation (Box and Cox 1964), selecting the optimal lambda from the log-likelihood function, while a log-transformation was used when  $-0.05 < \lambda < 0.05$ . A factorial

ANOVA including fertilization history, recent fertilization, measurement date, and their interactions as factors was not conducted due to persistent violation of the assumptions. Therefore, the effects of fertilization history, recent fertilization, and their interaction were tested separately for the measurement dates using a two-way ANOVA. Significantly different groups were identified using Tukey's HSD multiple comparison procedure in the case of significant main factor effects. User-defined linear contrasts with  $p$  values adjusted according to the joint  $t$  distribution (multcomp package, R statistics) were used in the case of significant interactions, thus investigating the effect of fertilization history for each of the recent fertilization treatments.

### 3.2.4 Model simulations

In addition to the investigation of fertilization history effects on the measured variables by means of ordinary statistics, a modeling approach was used to estimate soil C and N turnover rates in different soil components. The major aim was to obtain an estimate of gross turnover rates of C and N from the recently applied fertilizers, taking into account the variables that were measured in this study. By comparing the modeling results derived from two different model parameterizations, the effects of fertilization history on decomposition of recently added fertilizers was assessed. The procedure is described in detail below.

Organic matter C and N turnover were modeled using a conceptual pool model based on the soil-plant-atmosphere model Daisy (Abrahamsen and Hansen 2000). The model was modified by introducing two soil microbial residual (SMR) pools, following the suggestions of Mueller et al. (1998, 2003), who pointed out that upon microbial death not all the microbial N is immediately available as SMN, but some is temporally protected from decomposition. The model structure is presented in Fig. 3-1. In summary, added organic matter (AOM), soil microbial biomass (SMB), SMR, and previously existing, non-living soil organic matter (SOM) are each separated into two pools, one with a slow turnover rate (pool 1) and one with a fast turnover rate (pool 2), which are decomposed according to first-order kinetics. The turnover rate coefficients describe the rate at which C is transformed into other pools. The N flows are calculated according to pool-specific constant C/N ratios. Upon decomposition, matter from AOM, SMR, and SOM pools is utilized by the SMB pools, while the proportion of C respired to CO<sub>2</sub> is determined by conversion efficiencies that are specific to both the source and the SMB pool. Where the C flow from one pool is directed into two pools, partitioning coefficients are used to set the proportions of both flows. In the original Daisy

model, the turnover rate coefficients are modified by the actual soil temperature, soil water pressure potential, and in the case of SOM and SMB also by clay content. In the present model, the rate coefficients were corrected for soil temperature (22°C) while the effects of water and clay content were neglected since they were assumed identical in all treatments. The model comprised the mineralization-immobilization turnover and did not distinguish between  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ .

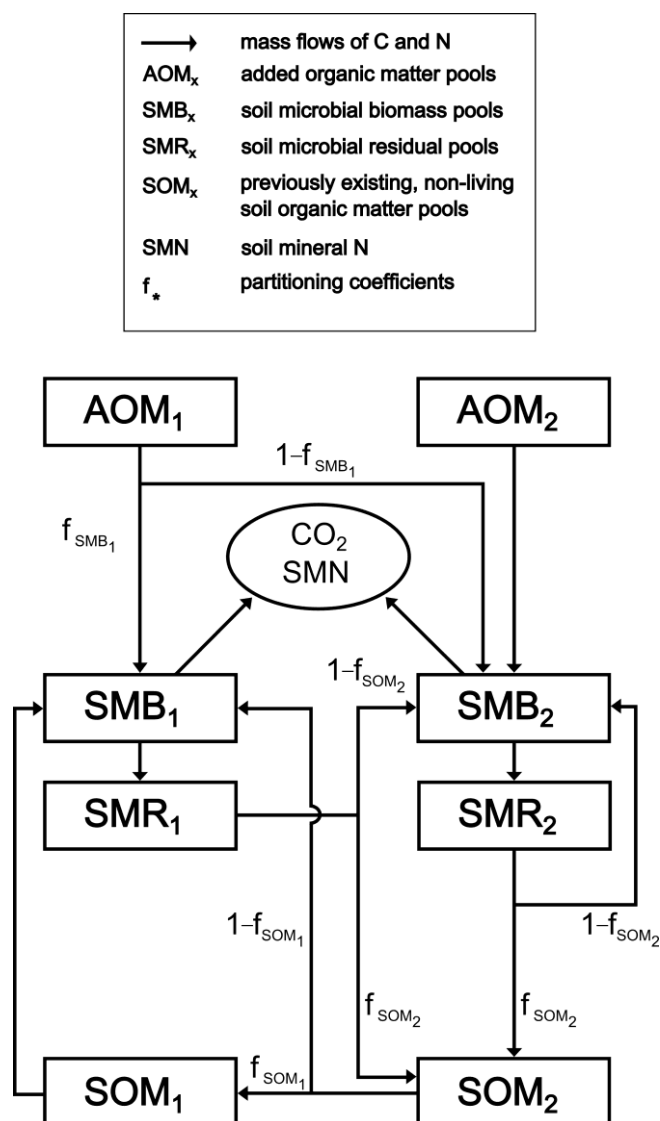


Figure 3-1: Part of the soil organic matter module of the Daisy model (Abrahamsen and Hansen 2000) that was used in this study, modified by introducing soil microbial residual pools ( $\text{SMR}_x$ ).

The model was used to simulate C and N dynamics during the incubation experiment. Initial values of total C and N in different pools of the model were set to measured values where available: measured C and N applied with the recent fertilization treatments were set to C and

N in AOM, measured soil total C and N were set to C and N in SOM, and microbial biomass C, determined at day 0 in the treatments without recent fertilization (RCO), was set to C in SMB. Soil mineral N was initialized using the SMN measurement at day 0 in the treatments without recent fertilization (RCO). In all treatments, default values implemented in Daisy were used for the parameters shown in Table 3-1. All other parameter values were estimated using a random search optimization algorithm. For the introduced SMR pools, both turnover rates were set to identical values. The best model fit was selected according to the smallest weighted residual sum of squares ( $wRSS$ ) between measured and simulated values of  $\Delta\text{CO}_2\text{-C}$ , SMN, and MBC. The reciprocal variances in measured values were used as weights to account for the differences in magnitude of the three measures.

Table 3-1: Default Daisy parameters (Hansen 2002) used in all model simulations.

Parameter	Value	Unit
EF SOM <sub>1</sub> by SMB <sub>1</sub>	0.4	g SMB-C (g C) <sup>-1</sup>
EF SOM <sub>2</sub> by SMB <sub>1</sub>	0.5	g SMB-C (g C) <sup>-1</sup>
EF SMR <sub>1/2</sub> by SMB <sub>2</sub> †	0.6	g SMB-C (g C) <sup>-1</sup>
f <sub>SOM1</sub>	0.1	-
f <sub>SOM2</sub> †	0.6	-
SOM <sub>2</sub> C per N	11.0	g C (g N) <sup>-1</sup>
SMB <sub>1/2</sub> C per N	6.7	g C (g N) <sup>-1</sup>
SMB <sub>1</sub> maintenance rate	0.0018	day <sup>-1</sup>
SMB <sub>2</sub> maintenance rate	0.01	day <sup>-1</sup>

† In the original Daisy model, matter from SMB pools is directly utilized by SMB<sub>2</sub> or transformed into SOM<sub>2</sub>. In the modified model of the present study, matter from SMB pools first enters the SMR pools and then is utilized by SMB<sub>2</sub> or transformed into SOM<sub>2</sub> (Fig. 3-1). We assumed that EF and the partitioning SMB<sub>2</sub>/SOM<sub>2</sub> with respect to SMR matter were the same as those for SMB matter in the Daisy model.

EF Carbon use efficiency of respective pool by SMB<sub>x</sub>

SMB<sub>x</sub> Soil microbial biomass pools (1: slow, 2: fast)

SOM<sub>x</sub> Previously existing, non-living soil organic matter pools (1: slow, 2: fast)

SMR<sub>x</sub> Soil microbial residual pools (1: slow, 2: fast)

Each simulation run included all 16 treatments and involved the fitting of all parameters simultaneously. The variation of parameters was restricted to a various number of groups, as described in the following. The SOM parameters (turnover rates, initial C/N ratio of SOM<sub>1</sub>, initial SOM<sub>1</sub>/SOM<sub>2</sub> partitioning) were fitted separately for the different fertilization histories, obtaining four different sets of parameters. The turnover rates of SMR, SMB<sub>1</sub>, and SMB<sub>2</sub> and the initial SMB<sub>1</sub>/SMB<sub>2</sub> partitioning were fitted once for all 16 treatments. For the AOM parameters (efficiencies, initial C/N ratios, initial AOM<sub>1</sub>/AOM<sub>2</sub> partitioning, turnover rates), we used two approaches. In the first simulation run, the AOM parameters were fitted by grouping the data according to the recent fertilization treatments (RFM, RPB, RCR), i.e. not allowing for possible effects of fertilization history on these parameters. In the second simulation run, the fitted AOM parameters from approach 1 were used as initial values and the whole simulation was repeated while fitting the AOM parameters individually for each of the 16 treatments, hence allowing for an effect of fertilization history on these parameters. The comparison of the modeling results derived from these two parameterization approaches with respect to the AOM parameters was used to assess fertilization history effects on the decomposition of recently applied fertilizers.

The three fitted C use efficiencies concerning AOM (AOM<sub>1</sub> by SMB<sub>1</sub>, AOM<sub>1</sub> by SMB<sub>2</sub>, and AOM<sub>2</sub> by SMB<sub>2</sub>) were used to calculate an overall C use efficiency (EF<sub>tot</sub>) of total SMB with respect to total AOM, considering the initial partitioning AOM<sub>1</sub>/AOM<sub>2</sub> and the fraction of AOM<sub>1</sub> that was directed to SMB<sub>1</sub>.

Model comparisons were performed using the information criterion proposed by Akaike (AIC; Akaike 1974) and corrected for sample size according to Hurvich and Tsai (1989). The criterion takes into account both the estimation error and the number of parameters fitted in the model. The corrected AIC (*AICc*) was calculated as:

$$AICc = n \ln(wRSS) + 2k + 2k(k + 1) / (n - k - 1),$$

where  $n$  is the number of observations,  $k$  is the number of independently fitted parameters in the model, and  $wRSS$  is the weighted residual sum of squares calculated as described earlier. The model with the smallest *AICc* is considered the best model.

### 3.3 Results

#### 3.3.1 Effects of recent fertilization, fertilization history, and their interactions

Recent fertilization significantly affected  $\Delta\text{MBC}$ ,  $\Delta\text{SMN}$ , and  $\Delta\text{CO}_2\text{-C}$  at all measurement dates (Table 3-2, Fig. 3-2). The effect of fertilization history depended on the variable investigated, as well as the measurement date, but was significant at the end of the incubation (day 147) for all three variables (Table 3-2). In the case of fertilization history effects on the decomposition of recently added fertilizer, the subject of this study, significant interactions between the factors fertilization history and recent fertilization were expected to be revealed in the two-way ANOVA. Such interactions were only detected in  $\Delta\text{SMN}$  at day 3 and 78 (Table 3-2). In this case, linear contrasts were computed in order to determine fertilization history effects for each of the recent fertilization treatments separately. These indicated that  $\Delta\text{SMN}$  at day 3 was significantly affected by fertilization history after recent fertilization with CR ( $\text{HFM} < \text{HCR} = \text{HPB} < \text{HCO}$ ) while in all other recent fertilization treatments there were no differences among the fertilization histories. At day 78,  $\Delta\text{SMN}$  was always lowest in the treatment HCO, highest in HFM, and intermediate in the treatments HPB and HCR, which were similar (Fig. 3-2). However, according to the linear contrast analysis, the treatments HPB and HCR could only be statistically separated from both other fertilization history treatments in the RPB treatment, not in RCO, RFM, or RCR. At day 147, the same trend ( $\text{HCO} < \text{HPB} = \text{HCR} < \text{HFM}$ ) was exhibited in  $\Delta\text{SMN}$  irrespective of recent fertilization treatment with no interactions between the main factors (Table 3-2, Fig. 3-2). This pattern ( $\text{HCO} < \text{HPB} = \text{HCR} < \text{HFM}$ ) was in concurrence with the initial values of MBC and SMN, which were a result of the differing long-term fertilization treatments applied in the field experiment (Fig. 3-3), as well as with soil total N and C contents, which were 0.49, 0.60, 0.63, and 0.95 g N kg<sup>-1</sup> dry soil and 6.2, 10.2, 7.7, and 11.1 g C kg<sup>-1</sup> dry soil in HCO, HPB, HCR, and HFM, respectively.

Table 3-2: Two-way ANOVA results ( $p$  values) for the effects of fertilization history (H), recent fertilization (R), and their interaction (H x R) on the net change in microbial biomass C contents ( $\Delta$ MBC), the net change in soil mineral N contents ( $\Delta$ SMN), and accumulated  $\text{CO}_2$ -C evolution ( $\Delta\text{CO}_2$ -C).

Measure	Effect	Days after start of incubation				
		0	3	9	78	147
$\Delta$ MBC	H	0.6515 †	0.5985 †	0.9270 †	0.5253	<b>0.0365</b>
	R	<b>&lt; 0.0001</b> †	<b>&lt; 0.0001</b> †	<b>&lt; 0.0001</b> †	<b>0.0065</b>	<b>0.0006</b>
	H x R	0.7732 †	0.1140 †	0.9918 †	0.6748	0.9855
$\Delta$ SMN	H	0.1081 †	<b>0.0044</b>	‡	<b>&lt; 0.0001</b> †	<b>&lt; 0.0001</b>
	R	<b>&lt; 0.0001</b> †	<b>&lt; 0.0001</b>	‡	<b>&lt; 0.0001</b> †	<b>&lt; 0.0001</b>
	H x R	0.2269 †	<b>&lt; 0.0001</b>	‡	<b>&lt; 0.0001</b> †	0.3410
$\Delta\text{CO}_2$ -C	H	n.a.	0.1706 †	0.3699 †	<b>0.0001</b> †	<b>0.0043</b>
	R	n.a.	<b>&lt; 0.0001</b> †	<b>&lt; 0.0001</b> †	<b>&lt; 0.0001</b> †	<b>&lt; 0.0001</b>
	H x R	n.a.	0.2737 †	0.2789 †	0.0594 †	0.2155

† Data transformed using a Box-Cox transformation.

‡ Prerequisites for ANOVA were not achieved at  $\alpha = 0.01$ .

$p$  values < 0.05 are shown in bold.

n.a. Not applicable

In  $\Delta$ MBC and  $\Delta\text{CO}_2$ -C, there were no interactions between the two main factors at any of the measurement dates. Hence, the observed differences among fertilization histories (Table 3-2, Fig. 3-2) did not depend on the recent fertilization treatment and vice versa. However, some main factor effects were discovered by the ANOVA. In  $\Delta$ MBC, the ANOVA indicated an overall effect of fertilization history at day 147 but the Tukey test did not detect any different groups. The values of  $\Delta\text{CO}_2$ -C, averaged across the recent fertilization treatments, differed between the fertilization history treatments according to ANOVA at days 78 and 147. According to the Tukey tests,  $\Delta\text{CO}_2$ -C was significantly lower in HCO than in the other fertilization histories at day 78, whereas at day 147, HCO and HCR were significantly lower than HPB, while HFM was in between and not separable from any treatment.



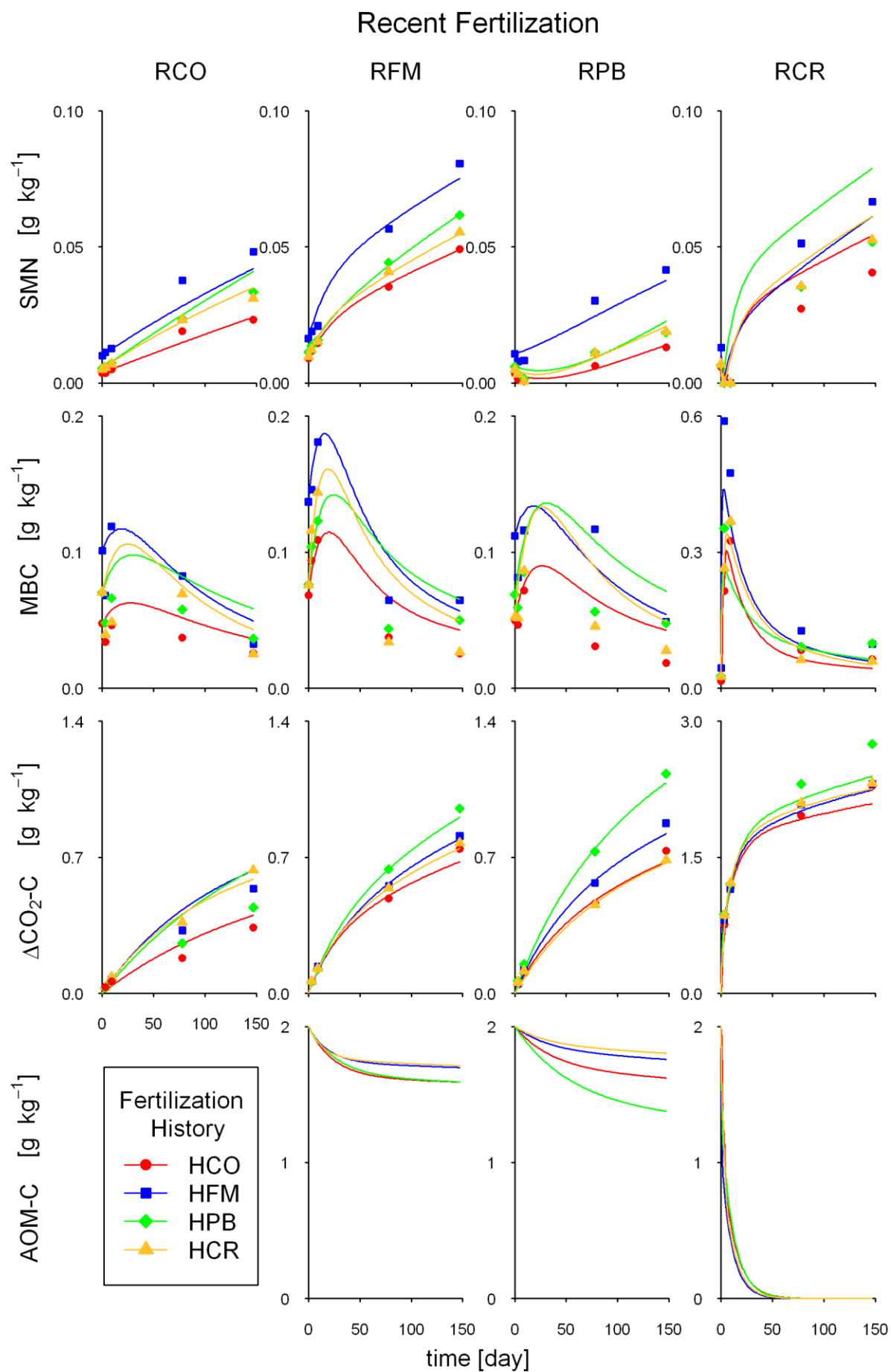


Figure 3-2: Measured (data points) and simulated (lines) soil mineral N contents (SMN; top row), soil microbial biomass C contents (MBC; second row), accumulated CO<sub>2</sub>-C release ( $\Delta$ CO<sub>2</sub>-C; third row), and added organic matter C (AOM-C; bottom row) in g kg<sup>-1</sup>. Columns represent recent fertilization treatments. Note the different scale of ordinates in the case of MBC and  $\Delta$ CO<sub>2</sub>-C. For sake of clarity, measured values of  $\Delta$ CO<sub>2</sub>-C were only presented for the measurement dates that were also available in SMN and MBC.

### 3.3.2 Model Simulations

When fertilization history effects were considered in the model, improvements were apparent in the overall agreement between measurements and simulations (comprising all measured variables, in all recent fertilization treatments) as indicated by smaller *wRSS* values (Table 3-3). The *AICc* not only evaluates the agreement between simulations and measurements, but also takes account of the number of independent parameters that need to be fitted. According to the *AICc* values, the fertilization history should be considered when modeling the turnover of FM and PB, however not when modeling the turnover of CR (Table 3-3).

The consideration of a fertilization history effect greatly improved the model simulations of  $\Delta$ CO<sub>2</sub>-C and SMN in the RPB treatment, however, for MBC there was no improvement but a slight decline (Table 3-3). In the RCR treatment, the simulated dynamics of MBC were improved when considering fertilization history and the initial net immobilization of N was well reflected by the model (Fig. 3-2). However, the initial peak in MBC of the HFM treatment was not predicted to the full extent by the model (Fig. 3-2). Also, at the end of the incubation, SMN values in the RCR treatment were overestimated by the model in all fertilization history treatments but HFM, while simulated  $\Delta$ CO<sub>2</sub>-C and MBC generally remained below the measurements at day 147 (Fig. 3-2). In contrast, for the RCO, RFM, and RPB treatments the simulation tended to overestimate MBC towards the end of the incubation (Fig. 3-2).

Table 3-3: Weighted residual sum of squares ( $wRSS$ ) and Akaike's information criterion ( $AICc$ ) for measured and simulated, accumulated  $\text{CO}_2$  release ( $\Delta\text{CO}_2\text{-C}$ ), microbial biomass C contents (MBC), and soil mineral N contents (SMN) as affected by recent fertilization and the consideration of fertilization history effects in the model.

Recent Fertilization	Fertilization History Effect	Dependent Variable (sample size)					Parameters†
		$\Delta\text{CO}_2\text{-C}$ (588)	MBC (16)	SMN (16)	All data‡ (620)	All data‡ (620)	
		$wRSS$				$AICc$	$k$
RFM	considered	0.4	0.7	0.2	1.4	298	48
	not considered	0.9	0.9	1.0	2.8	698	24
RPB	considered	0.5	1.0	0.3	1.8	462	48
	not considered	4.4	0.8	1.7	6.9	1250	24
RCR	considered	13.6	5.6	6.5	25.7	2118	48
	not considered	12.9	9.7	4.8	27.4	2103	24

<sup>†</sup> Number of independently fitted model parameters

<sup>‡</sup> Data of  $\Delta\text{CO}_2\text{-C}$ , MBC, and SMN weighted and pooled

RFM, RPB, RCR Recent fertilization with farmyard manure, pine bark, and cabbage crop residues, respectively

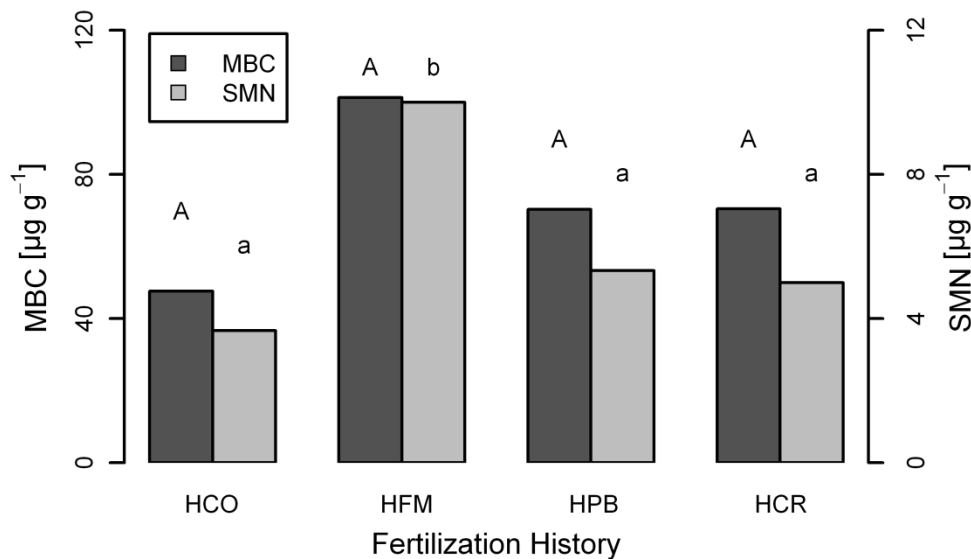


Figure 3-3: Initial values (measurement date 1, RCO) of microbial biomass C (MBC) and soil mineral N (SMN) contents in  $\mu\text{g g}^{-1}$  dry soil. Different letters above the columns indicate significant ( $p < 0.05$ ) differences between fertilization history treatments in MBC (upper case) and in SMN (lower case), respectively.

In the model simulations, the white cabbage material recently added in RCR was almost completely decomposed 50 days after the start of incubation in all fertilization history treatments (Fig. 3-2). The simulated decomposition of FM was much slower but also very similar among the fertilization histories, accounting for 14 (HCR), 15 (HFM), and 20% (HPB, HCO) of the added C mineralized at the end of the incubation (Fig. 3-2). In contrast, there were distinct differences in the simulated decomposition of PB, which at the end of the incubation constituted 10 (HCR), 12 (HFM), 18 (HCO), and 31% (HPB) of the added C (Fig. 3-2).

The corresponding parameters used in the model simulations are presented in Table 3-4. The higher simulated C mineralization from PB in the HCO and in particular in the HPB treatment compared to the other fertilization history treatments was reflected in the model parameters by higher initial fractions of the fast AOM pools ( $AOM_2$ ; Table 3-4) and faster turnover rates of the slow AOM pools ( $AOM_1$ ; Table 3-4). In contrast, the C use efficiencies of total AOM with respect to total SMB ( $EF_{tot}$ ) concerning pine bark (RPB) were estimated to decrease in the order  $HCR > HPB > HFM > HCO$  (Table 3-4).

Table 3-4: Parameters fitted in the model simulations considering fertilization history effects.

Parameter	Value			Unit
	<u>Common for all treatments</u>			
SMB <sub>2</sub> initial fraction	0.04			-
SMB <sub>1</sub> turnover rate	0.024			day <sup>-1</sup>
SMB <sub>2</sub> turnover rate	0.024			day <sup>-1</sup>
SMR <sub>x</sub> turnover rate	0.0018			day <sup>-1</sup>
	<u>Fertilization history treatments: HCO / HFM / HPB / HCR</u>			
SOM <sub>2</sub> initial fraction	0.200 / 0.146 / 0.198 / 0.187			-
SOM <sub>1</sub> turnover rate	5.0 / 5.7 / 0.9 / 8.1			10 <sup>-7</sup> day <sup>-1</sup>
SOM <sub>2</sub> turnover rate	3.3 / 4.8 / 3.2 / 5.3			10 <sup>-3</sup> day <sup>-1</sup>
SOM <sub>1</sub> C per N †	13 / 12 / 20 / 13			g C (g N) <sup>-1</sup>
<u>Recent fertilization treatment:</u>	<u>RFM</u>	<u>RPB</u>	<u>RCR</u>	
EF <sub>tot</sub> †	0.41 / 0.46 / 0.43 / 0.44	0.26 / 0.29 / 0.31 / 0.35	0.17 / 0.22 / 0.14 / 0.19	g SMB-C (g C) <sup>-1</sup>
AOM <sub>1</sub> fraction to SMB <sub>1</sub>	0.52 / 0.52 / 0.45 / 0.44	0.58 / 0.63 / 0.63 / 0.61	0.69 / 0.75 / 0.72 / 0.68	-
AOM <sub>2</sub> initial fraction	0.19 / 0.13 / 0.18 / 0.12	0.16 / 0.09 / 0.30 / 0.07	0.42 / 0.48 / 0.34 / 0.46	-
AOM <sub>1</sub> turnover rate	7.4 / 7.2 / 8.6 / 8.9	13 / 12 / 16 / 10	3347 / 3990 / 3556 / 3764	10 <sup>-5</sup> day <sup>-1</sup>
AOM <sub>2</sub> turnover rate	1.8 / 2.3 / 1.6 / 2.9	1.2 / 1.4 / 0.8 / 1.4	29 / 35 / 34 / 22	10 <sup>-2</sup> day <sup>-1</sup>
AOM <sub>1</sub> C per N †	12 / 14 / 12 / 13	137 / 126 / 135 / 124	28 / 38 / 28 / 28	g C (g N) <sup>-1</sup>
AOM <sub>2</sub> C per N	11 / 6 / 12 / 8	75 / 85 / 97 / 89	28 / 22 / 28 / 28	g C (g N) <sup>-1</sup>

SMB<sub>x</sub> Soil microbial biomass pools (1: slow, 2: fast)

SMR<sub>x</sub> Soil microbial residual pools

SOM<sub>x</sub> Previously existing, non-living soil organic matter pools (1: slow, 2: fast)

EF<sub>tot</sub> Carbon use efficiency of total SMB with respect to total AOM

AOM<sub>x</sub> Added organic matter pools (1: slow, 2: fast)

† Not individually fitted but derived from other fitted and default parameters.

HCO / HFM / HPB / HCR Long-term fertilization with nothing (HCO), farmyard manure (HFM), pine bark (HPB), or crop residues (HCR).

RFM, RPB, RCR Recent fertilization with farmyard manure (RFM), pine bark (RPB), or cabbage crop residues (RCR)

## 3.4 Discussion

### 3.4.1 Effects of fertilization history on decomposition of recently added fertilizer

Long-term fertilization with organic substrates differing greatly in their physicochemical properties had a temporary effect on N mineralization from recently added organic matter, as indicated by the significant interactions between recent fertilization and fertilization history in  $\Delta$ SMN found at day 3 and 78 (Table 3-2). However, at the end of the incubation there were no significant interactions and interestingly the differences in  $\Delta$ SMN among the fertilization history treatments at days 78 and 147 showed the same pattern as in soil total N and C contents, as well as initial MBC and SMN values (Fig. 3-2 and Fig. 3-3). This trend was observed across all recent fertilization treatments, including the control (RCO). Hence, fertilization history effects on net N mineralization were presumably mainly derived from differences in the N mineralization from previously existing SOM, at least regarding the period after the first two weeks. This result was consistent with those of Hadas et al. (1996), who found that within the period of 32 weeks, net N mineralization from cattle manure was not higher in soils with long-term cattle manure fertilization compared to soils that had not been fertilized for 30 years. The authors argued that the decomposition of recently added organic matter may be independent of the initial soil microbial biomass and activity. Similarly, Langmeier et al. (2002) found no consistent effect of fertilization history (organic vs. conventional) on plant N uptake from recently added cattle feces and slurry, although the soils used differed greatly in soil microbial biomass. In both studies, differences in net N mineralization were mostly due to differences in N mineralization from previously existing SOM.

In accordance with the results of Fauci and Dick (1994), the microbial biomass of the different soils did not respond differently to the recent fertilization treatments, essentially maintaining the initial pattern present at day 0 in the RCO treatment: HFM > HPB = HCR > HCO (Fig. 3-2 and 3-3). Hence, the microbial communities were able to adapt to all organic fertilizers, irrespective of fertilization history. Ruppel et al. (2007) investigated the soils also used in the present study and analyzed the prokaryotic potential functional diversity, using community-level physiological profiling, and the phylogenetic diversity, using polymerase chain reaction-denaturing gradient gel electrophoresis in combination with sequencing analysis. Their results showed that with increasing soil N availability the prokaryotic

functional diversity of the soil decreased, which was associated with a shift in community composition. However, this observed decrease in functional diversity and shift in community composition in the HFM treatment, compared to HCO and HPB (Ruppel et al. 2007), apparently did not affect N mineralization from recently added organic matter, at least over a period of months. This finding suggests that initial differences in the functional diversity and community composition of the prokaryotes were either not relevant for N mineralization of recently added fertilizer or not sustained over the incubation period. The latter would agree with the observed capability of soil microorganisms to quickly adapt to a new nutrient source as demonstrated by shifts in substrate utilization patterns and community fatty acid methyl ester profiles within days after incorporation of different C sources (e.g. Schutter and Dick 2001).

Another effect that may have compensated for initial differences in microbial biomass and microbial community structure is the direct input of microorganisms present in the recently applied fertilizers. It can be assumed that microorganisms already inhabiting the fertilizers are well adapted to these substrates. Hence, the existence of microorganisms in the soil capable of decomposing the recently added fertilizers is not a prerequisite for their decomposition. However, Nett et al. (2010) reported that net N mineralization from recently applied farmyard manure was about twice as high after 10 weeks in a soil with a long-term history of farmyard manure fertilization compared to non-fertilized soil. Also Mallory and Griffin (2007) observed a fertilization history effect, but in their study the availability of N from recently applied manure was reduced in soil fertilized with organic fertilizer in the past compared to inorganically fertilized soil. The authors attributed this to a more active microbial community in the organically fertilized soil, capable of immobilizing more N. These contrasting results can perhaps be attributed to the different experimental approaches used in these studies. Compared to the highly controlled conditions in the laboratory study of Mallory and Griffin (2007), the study by Nett et al. (2010) involved a greenhouse experiment with pot-grown plants, which may have resulted in an indirect effect of fertilization history on decomposition of recently applied fertilizer, e.g., by feedback effects through modified plant growth and associated changes in rhizodeposition.

On the other hand, Stark et al. (2008) applied lupin (*Lupinus angustifolius* L.) residues to soils with a long-term history of organic vs. conventional fertilization and found that the response with respect to microbial biomass increase, enzyme activities, and net N mineralization was very similar, although the soils differed considerably in microbial community structure. The

latter study may be interpreted as a support of the findings of the present study, viz. that there was no fertilization history effect on MBC response and N mineralization after recent application of organic fertilizers.

Finally, there was no evidence that fertilization history had an effect on the net C mineralization from recently added organic fertilizers differing greatly in their physicochemical properties, since no interactions between the effect of fertilization history and recent fertilization were found at any of the measurement days.

### 3.4.2 Model simulations

Using the Daisy model, Mueller et al. (1998, 2003) observed systematic overestimations of SMN upon decomposition of microbial biomass. They attributed this to the insufficient differentiation of organic matter and suggested the introduction of SMR pools with turnover rates between those of SOM and SMB pools. Similarly, in the present study, the simulations with the original Daisy approach showed a considerable overestimation of SMN in the RCR treatment and a minor one in the other recent fertilization treatments (data not shown). When SMR pools were included, a systematic overestimation of SMN after the initial peak in SMB in the RCR treatment (Fig. 3-2) was still observed but the overestimation was considerably smaller as compared to the original Daisy approach. Therefore, the results were interpreted as evidence for the validity of the inclusion of SMR pools in the conceptual model.

As indicated by the values of  $wRSS$  and  $AICc$ , considering a fertilization history effect on the turnover of AOM, by using turnover rates, substrate use efficiencies, and fractionation coefficients specific to both substrate and soil, clearly improved the model fit in the treatments RFM and RPB, however not in RCR (Table 3-3). Hence, the inclusion of fertilization history effects on the above-mentioned parameters may be justified, despite the increased number of independent parameters needing to be fitted being considered a penalty according to the  $AICc$ . The improvement was most apparent for  $\Delta CO_2\text{-C}$  and SMN in the RPB treatment, which corresponded to the faster simulated decomposition of PB in soil that had always received pine bark compared to the other soils (Fig. 3-2). Together with the differences in the parameter estimates, these results may be interpreted as a sign of increased microbial capability to access pine bark compared to the other fertilization histories, whereas there was no evidence of considerable changes in C use efficiencies. The apparent contradiction between the results of the model fitness ( $AICc$ ) and the results of the measurements alone (ANOVA), can be attributed to the fact that the  $AICc$  does not account



for variability in measurements. The *AICc* provides a definite value without a confidence interval, which does not allow to draw conclusions about the statistical significance of the result. Moreover, the degree of penalty for additional independent model parameters when using the *AICc* has been questioned by some authors (e.g. Bozdogan 2000), attenuating the validity of the *AICc* results.

The estimated overall substrate use efficiencies of 0.14–0.46 g MBC g<sup>-1</sup> substrate-C ( $EF_{tot}$ , Table 3-4) were in the range reported by Mueller et al. (1997). The authors argued that the Daisy default value of 0.6 g MBC g<sup>-1</sup> substrate-C (Hansen 2002), irrespective of the SMB and AOM pool, is generally too high. They also suggested that the substrate use efficiency of more recalcitrant organic matter should be lower than that of easily decomposable matter. The latter proposition was only partly confirmed by the results of the present study, with substrate use efficiency being higher for FM compared to PB. However,  $EF_{tot}$  was even lower for CR although CR exhibited by far the fastest turnover (Table 3-4). This may be linked to the systematic underestimation of MBC during the early stages of CR decomposition (Fig. 3-2).

The contrasting degradability of the applied fertilizers (FM, PB, CR) was well reflected in the distinct fractions of AOM-C decomposed at the end of the five-months incubation (Fig. 3-2, bottom row). It was obvious that in the RCR treatment potential differences among fertilization histories could only be revealed in a time span of about one month, since after 50 days the fertilizer was almost completely decomposed in all treatments. In contrast, detecting potential fertilization history effects on the decomposition of FM and PB was unlikely within one month after application, since only about 20% of the added C had been decomposed by this time. This demonstrates the major importance of the duration of the experiment when determining potential fertilization history effects on the decomposition of recently applied fertilizers. Also, it should be noted that due to the favorable abiotic conditions, turnover of organic matter proceeds much faster in laboratory incubation experiments than in the field. According to a model by Franko and Oelschlägel (1995), the effective mineralization time under field conditions corresponding to those at the Großbeeren site could be as high as seven times the actual incubation time. Hence, the 147-day incubation experiment likely represents a period of actual mineralization in the field that is much longer than the duration of the incubation experiment.

### **3.4.3 Conclusions**

In conclusion, fertilization history effects should be considered when modeling the turnover of recently added organic matter in the case of pine bark and farmyard manure, not however in the case of easily decomposable cabbage material. Hence, the hypothesis that fertilization history effects on the decomposition of recently added fertilizer depend on the type of fertilizer was confirmed. However, the results further indicated that effects of long-term fertilization history on net C and N mineralization from recently applied organic fertilizer can be neglected for fertilization practice when considering a period of five months under optimal abiotic conditions for microbial growth. Even in soil that had not received any fertilizer for 35 years, the net N mineralization of recently applied fertilizers after five months was not different from treatments fertilized with contrasting organic fertilizers each year since 1973. However, the investigated fertilization history effects did not include indirect effects of modified SOM contents on turnover rates by means of altered soil temperature, moisture, or aeration, nor effects of soil fauna, soil structure or the macro-physical properties of the used fertilizers, since these were ground before application. Such factors have an influence in the field but have a minimal or no effect under the conditions of the presented laboratory experiment.

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## 4 Effect of winter catch crops on nitrogen surplus in intensive vegetable crop rotations

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### Abstract

The nitrogen (N) use efficiency of field vegetable production systems needs to be increased in order to, reduce the detrimental effects of N losses on other ecosystems, save on production costs, and meet the limits set by the German government concerning N balance surpluses. Winter catch crops (CCs) have been shown to be a useful tool for reducing N losses in many agricultural production systems. This study was designed to test the effects of different CCs: rye (*Secale cereale* L.), fodder radish (*Raphanus sativus* L. var. *oleiformis* Pers.), bunch onion (*Allium cepa* L.), and sudangrass (*Sorghum sudanense* Stapf), planted at different sowing dates (early, late), on the N balance of 2-year vegetable crop rotation systems. The crop rotations started with a cauliflower (*Brassica oleracea* L. var. *botrytis* L.) crop, which was fertilized with N in a conventional manner. The experiments took place at three different sites in Germany. Results revealed that the average N balance surplus, when taking into consideration, fertilization, soil mineral N, and aboveground plant biomass N, was 217 kg N ha<sup>-1</sup> in the control treatments without a CC. This high value was mainly a consequence of large quantities of crop N and soil mineral N remaining after the harvest of the cauliflower. In spite of these high N surpluses, the application of CC only reduced the N balance surplus, on average across all sites and experiments, by 13 kg N ha<sup>-1</sup>, when compared to the control treatments. The type of CC and the sowing date had only minor effects on the N balance. The findings of this study suggest that for many sites the application of CCs does not solve the problem of high N balance surpluses in intensive field vegetable production systems.

### Keywords

catch crop, cover crop, nitrogen balance, nitrogen surpluses, crop rotation, nitrogen fertilization

## 4.1 Introduction

Field vegetable production systems are particularly prone to nitrogen (N) losses from the soil-plant system for several reasons. Firstly, the fertilizer recommendations of most vegetable crops suggest that soil mineral N (SMN) content should be maintained up until harvest at a level that is at least 40 kg N ha<sup>-1</sup> in excess of the expected crop N uptake (Feller et al. 2010). The reason for this is that harvest usually takes place during the reproductive stage of the crop when the N demand is very high, and also, because N limitation is associated with a reduction in quality of the freshly sold product. Secondly, many vegetable crops, especially *Brassica* species such as cauliflower (*Brassica oleracea* L. var. *botrytis* L.), broccoli (*Brassica oleracea* L. var. *italica* Plenck), and Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* (DC.) Zenker), leave more than 150 kg N ha<sup>-1</sup> in crop residues after harvest (Feller et al. 2010), which upon decomposition can be lost via leaching or emission of nitrogenous gases (Rahn et al. 1992). Thirdly and finally, vegetable root crops are preferably cultivated on coarse-textured soils (Simonne and Hochmuth 2003), which are particularly susceptible to N leaching losses (Kurunc et al. 2011).

High N losses result in increased fertilizer application rates and thus higher financial cost to the farmer. In addition, N losses cause negative environmental effects such as, the contribution of nitrogenous gases to global warming (Vitousek et al. 1997), the eutrophication of ecosystems (Smith et al 1999), and increased concentrations of nitrate in drinking water (Fan and Steinberg 1996). In order to reduce nitrate leaching from agricultural soils, The European Union introduced the “Nitrates Directive” (EC 1991) in 1991. In response to this instruction, The German Fertilizer Ordinance imposed limits to N balance surpluses at the field scale. These limits have been lowered gradually over the last four years and are at present set to 60 kg N ha<sup>-1</sup> yr<sup>-1</sup> for an area-weighted average of three consecutive years and one farm. To meet these regulations, whilst maintaining the competitiveness of the industry, new farming strategies are needed.

A promising approach for reducing N losses, is the inclusion of catch crops (CCs) in vegetable crop rotations. In theory, CCs take up N that would otherwise be lost, and, after incorporation of the CC residues into the soil, make this N available to the succeeding crop. However, the influence of a CC on the supply of N to the succeeding crop can vary greatly and range from a positive to a negative effect. The effect is positive when the fraction of N

mineralized from CC residues is greater than the fraction of N in the CC that would have been retained in the soil without a CC (Thorup-Kristensen and Nielsen 1998). The key factors determining these fractions are, the SMN availability after harvest of the preceding crop, N leaching during the time between preceding and succeeding crop, N uptake by the CC, mineralization rate of CC residues, and rooting depth of the succeeding crop. Christiansen et al. (2006) and Thorup-Kristensen (2001) highlight the N uptake capacity and the rooting depth of a CC as the crucial factors under conditions of high leaching rates. Since the prevention of leaching by a CC only has a positive effect when the N delivered from the CC residues to the succeeding crop would have not been available to the succeeding crop without a CC, a shallow-rooted succeeding crop generally benefits more from CC use than a deep-rooted succeeding crop (Thorup-Kristensen 2006). All of these factors are influenced by the weather and soil conditions. In addition, mineralization of organic residues, such as the residues of the preceding crop and the CC, is mainly determined by their physicochemical properties (Cabrera et al. 2005). Apart from the weather conditions, which cannot be controlled, management practice can be altered to enhance the benefits of the CC, mostly by adjusting crop species choice and rotation, sowing and harvesting dates of crops, and soil tillage techniques.

The average N balance surplus, calculated as the difference in N input (fertilization, atmospheric deposition, biological N fixation, drilling/transplanting) and N output (crop harvest), for the total agricultural area of Germany was estimated to constitute on average 101 kg ha<sup>-1</sup> yr<sup>-1</sup> in the years 1996–2006 (BMU and BMELV 2008, p.30). Despite being a useful value, this average fails to indicate the great spatial variability in N balance surpluses that occurs depending on the production system and management practices used. For field vegetable production systems the average surpluses are much higher, for the reasons mentioned earlier, especially when *Brassica* crops are involved. According to a comprehensive data set, compiled by Feller et al. 2010 in order to support the fertilizer recommendation systems for German field vegetable production, the recommended N fertilization exceeds the amount of N in the harvested product in many cases. This excess is 50–100 kg ha<sup>-1</sup> in 36% of cases and more than 100 kg ha<sup>-1</sup> in 19% of cases for 47 of the most common vegetable crops (Feller et al. 2010). Despite this imbalance in N, CCs are still not widely applied to these systems. This is in part due to a gap in the knowledge about suitable CC strategies that can reduce N losses without negative impacts on farm income.

The aim of this study was to find appropriate CC strategies for typical German intensive vegetable farming systems. Three separate locations were selected to represent a range of soil and weather conditions. The hypothesis tested was that CC use, compared to a fallow period during winter, can significantly increase the recovery of N at the end of a two-year vegetable crop rotation by an amount that is relevant to fertilization practice (i.e.  $> 30 \text{ kg N ha}^{-1}$ ).

## **4.2 Materials and Methods**

### **4.2.1 Experimental sites**

Field experiments were performed at three different sites in Germany: Großbeeren (13.31°E, 52.35°N), 20 km south of Berlin, Golzow (14.49°E, 52.57°N), 30 km north of Frankfurt/Oder, and Schifferstadt (8.35°E, 49.40°N) in the Upper Rhine Plain, 20 km southwest of Mannheim. The sites greatly differed in soil properties and climatic conditions (Table 4-1). Due to these differences, typical vegetable crop rotations vary according to location. In Großbeeren and Golzow vegetable crops account for 1/3 of the rotation and agricultural crops 2/3, whilst in Schifferstadt the crop rotation is exclusively made up of vegetable crops. Because of differences in soil texture and the distribution of heavy rainfall, the risk of N leaching can be expected to decrease in the order: Großbeeren > Schifferstadt > Golzow. Also, the low mean annual temperature and high number of annual frost days excludes the use of thermophilic CCs in Golzow.

Table 4-1: Soil properties and climate conditions at the experimental sites.

Property	Unit of measurement	Site		
		Großbeeren	Golzow	Schifferstadt
Soil type	[-]	Arenic Luvisol	Fluvic Cambisol	Haplic Luvisol
soil pH (0.01 M CaCl <sub>2</sub> ) <sup>a</sup>	[-]	6.6	6.7	7.5
Organic C content <sup>a</sup>	g kg <sup>-1</sup>	7.0	12.8	9.4
Sand / Silt / Clay <sup>a</sup>	grav. %	91 / 4 / 5	47 / 25 / 28	34 / 47 / 19
Annual precipitation sum <sup>b</sup>	mm yr <sup>-1</sup>	500	513	531
Mean Annual temperature <sup>b</sup>	°C	9.8	9.6	11.5
Number of frost days <sup>b, c</sup>	[1]	38	44	23

<sup>a</sup> Soil depth interval 0–30 cm<sup>b</sup> Average of the years 1999–2009<sup>c</sup> Days with average temperature < 0°C

## 4.2.2 Crop rotation experiments

At each experimental site, crop rotation experiments were performed that lasted approximately 1.5 years and comprised, a preceding crop in the summer of the first year, a CC over the winter, and a succeeding crop during the summer–autumn period of the next year. The preceding crop was cauliflower in all cases. To account for variability in weather conditions, these experiments were set up on independent plots over three consecutive years (2006–2007, 2007–2008, and 2008–2009) at the Großbeeren and Golzow sites and over two consecutive years (2007–2008, 2008–2009) at the Schifferstadt site. Two experimental factors were tested: “factor date” and “factor CC”. Factor date referred to the harvesting date of the preceding crop and sowing date of the CC, and had two levels: “early” and “late”, being approximately two weeks apart. Factor date could not always be realized and so, in the first experiment at the Golzow site and in the second experiment at the Großbeeren site, there was an early and a late set of cauliflower but the CCs were sown at identical dates. At the Schifferstadt site in both experiments, only the CCs were grown two weeks apart, the cauliflower crops were cultivated simultaneously. Factor CC represented the type of CC with either, no CC (control), a cold-hardy CC, or a non-cold-hardy CC being used. The experimental design was a split-plot with four replications at the Großbeeren and Schifferstadt sites and three at the Golzow site. Factor date was represented by the main plots and

randomized within replication blocks. Factor CC was represented by the subplots. The CCs used at the Großbeeren and Golzow sites were cold-hardy winter rye (*Secale cereale* L.) and non-cold-hardy fodder radish (*Raphanus sativus* L. var. *oleiformis* Pers.), whilst at the Schifferstadt site, they were cold-hardy bunch onion (*Allium cepa* L.) and non-cold-hardy sudangrass (*Sorghum sudanense* Stapf). In the second experiment, the sudangrass that was sown late had to be replaced by winter rye. All CCs, bar the bunch onion, were completely incorporated into the soil as a green manure (sudangrass in autumn, other CC in spring). The bunch onion was harvested conventionally and only the crop residues were incorporated into the soil (in spring). At the Großbeeren and Golzow sites, the succeeding crop in the first two experiments was beetroot (*Beta vulgaris* L. subsp. *vulgaris*) and in the third, spinach (*Spinacia oleracea* L.). In the first experiment the establishment of the beetroot crop at the Großbeeren site failed due to unfavorable weather conditions. At the Schifferstadt site, the succeeding crop was celeriac (*Apium graveolens* L. var. *rapaceum* (Mill.) DC.) in both experiments. The time schedules of all crop rotations are depicted in Fig. 4-1.

#### 4.2.3 Sampling and chemical analyses

From each treatment plot, plant and soil samples were taken periodically on a monthly basis from individual sample plots of 1–2 m<sup>2</sup>, separated by a strip of at least 0.25 m width. Sampling of aboveground plant biomass involved completely harvesting the sample plots and separating the different plant organs into leaf, stem, storage organ, and reproductive organ fractions. Total C and N contents in the plant dry matter (dried at 60°C) were determined using a CNS-Analyzer VARIO EL (Elementar, Hanau, Germany).

Per sample plot, 16 soil cores (inner diameter 1.8 cm) were taken and pooled to obtain one composite sample, which was then separated into three soil depth sections (0–30, 30–60, and 60–90 cm). For contents of SMN ( $\text{NO}_3^-$ -N +  $\text{NH}_4^+$ -N), a dry mass equivalent of 25 g of moist soil was extracted with 100 ml of 0.0125 M  $\text{CaCl}_2$  solution and concentrations of  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N were determined colorimetrically in the extract using an EPOS analyzer (Eppendorf, Hamburg, Germany).



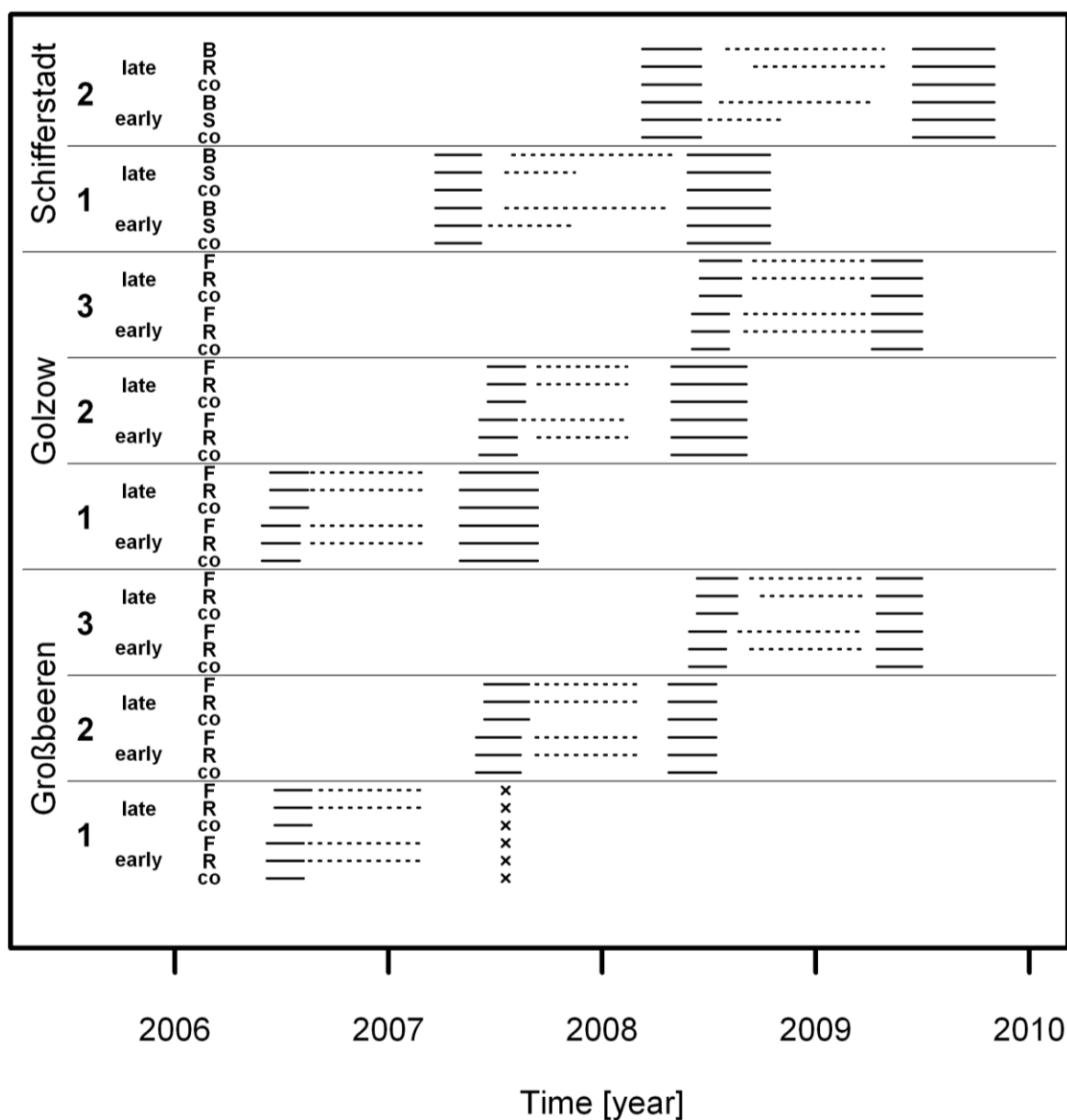


Figure 4-1: Time schedules of all crop rotations. Solid lines: preceding cauliflower crops and succeeding crops, respectively, dashed lines: catch crops. Catch crop treatments: “co” fallow control, “R” winter rye, “F” fodder radish, “S” sudangrass, “B” bunch onion. “x” end of considered crop rotation after establishment of succeeding crop failed in the first experiment at the Großbeeren site.

#### 4.2.4 Calculation of N balances

A balance of N for each crop rotation experiment was calculated on the basis of N input into, and N output out of, the crop rotation. The input was defined as the sum of, N in above-ground crop residues of the cauliflower crop, SMN in the depth section 0–90 cm at time of cauliflower harvest, and mineral N fertilization between cauliflower harvest and harvest of the succeeding crop (always calcium ammonium nitrate). Hence, the period used for N balance calculations did not include the cauliflower crop. This approach was chosen because, the amount of N in cauliflower crop residues was highly variable, and, the time of cauliflower harvest was the most appropriate date to determine the total N input comprising both SMN and crop residues. The N output was defined as the sum of, N in aboveground plant biomass of the succeeding crop at harvest, SMN in the depth section 0–90 cm at this time, and in the case of bunch onion, the amount of N harvested with the CC. In the first experiment at the Großbeeren site, where the cultivation of the succeeding crop failed, the N output was exclusively comprised of SMN contents in the summer of the second year (2007). The difference in N input minus N output was referred to as the apparent N loss. To assess the impact of the CC, the difference between the apparent N loss of a certain CC treatment minus that of the corresponding control treatment, i.e. the treatment without a CC at the same site and sowing date and for the same experiment, was calculated. Henceforth, this difference will be termed CC effect.

The maximum aboveground N content of the CC biomass was used to relate the CC N uptake to the CC effect. Hence, in the case of part or complete frost kill of the CC during winter, the last measurement of N contents of the undamaged crop was used.

In the second experiment at the Golzow site, the N output exceeded the input, on average by 44% ( $114 \text{ kg N ha}^{-1}$ ). This result was probably caused by plant uptake of SMN from unsampled soil depths (deeper than 90 cm), or, of N mineralized from soil organic matter, which was not considered in the balance calculations. Therefore, this data set was omitted from the analyses.

In addition to the approach described above, the N balances were also calculated as prescribed by The German Fertilizer Ordinance, i.e. only considering N fertilization and removal of N with crop harvest, not SMN. These calculations comprised the whole crop rotation, including the preceding cauliflower crop and the succeeding crop of the second year. To obtain a yearly average of N balance surplus, results were divided by two.

#### 4.2.5 Statistics

Statistical analyses were performed using R statistics (v. 2.11.1; R Development Core Team, 2010). For parametric tests, the assumption of normal distribution of within-group errors was tested by the Kolmogorov–Smirnov test, while homoscedasticity was checked using the Levene’s test. Unless stated otherwise, results are presented as a mean  $\pm$  1 standard error of the mean. Statistical significance was stated at  $p < 0.05$ .

### 4.3 Results

#### 4.3.1 Nitrogen balance

On average across all sites, experiments, and treatments, the N input into the crop rotations amounted to  $386 \pm 8 \text{ kg ha}^{-1}$  (Fig. 4-2). About half of this input could be attributed to cauliflower crop residues, one quarter to the initial SMN content (from the soil depth section 0–90 cm; Fig. 4-2), and the final quarter to N from mineral fertilization (not shown). Of the total N input, on average 47% was recovered in the total N output, corresponding to an apparent absolute N loss of  $203 \pm 8 \text{ kg ha}^{-1}$  ( $n = 139$ ). In the treatments with onion as the CC, the average amount of N removed with onion CC harvests was  $88 \pm 6 \text{ kg ha}^{-1}$ , constituting 30% of the total N output in these treatments ( $n = 16$ ). Averaged across all treatments that comprised a succeeding crop, i.e. with the exception of the first experiment at the Großbeeren site, the amount of N in aboveground biomass of the succeeding crops was  $127 \pm 3 \text{ kg ha}^{-1}$  ( $n = 118$ ), which constituted 70% of the total N output in these treatments.

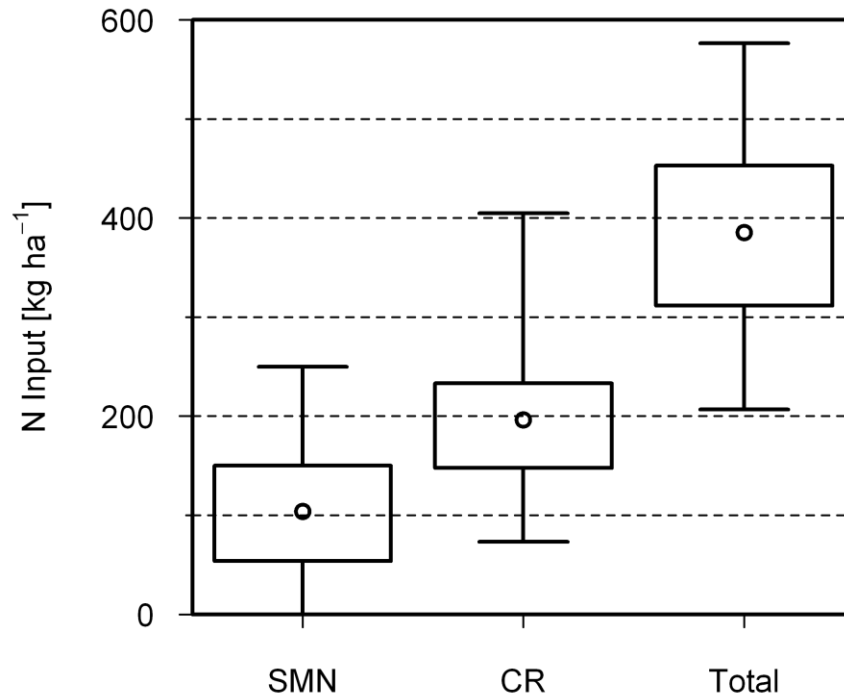


Figure 4-2: Nitrogen inputs of the experimental crop rotations in  $\text{kg ha}^{-1}$ , separated into different components, SMN: soil mineral N in soil depth section 0–90 cm, CR: N in crop residues of preceding cauliflower crop, Total: sum of N in SMN, CR, and mineral fertilization (not shown). For each component, sample size = 139. Circle: arithmetic mean, hinges: 25% and 75% quantile, whiskers: minimum and maximum values.

The balances calculated according to The German Fertilizer Ordinance exhibited an average N surplus of  $55 \pm 6 \text{ kg ha}^{-1} \text{ yr}^{-1}$  ( $n = 36$ ), hence remaining  $5 \text{ kg ha}^{-1} \text{ yr}^{-1}$  below the permitted surplus. Still, the calculated N balance surpluses exceeded the limit of  $60 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in 56% of cases, and a value of  $80 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in 45% of cases.

#### 4.3.2 Catch crop effects on apparent N losses

On average for all treatments, the CC effect (Fig. 4-3) was  $-13 \pm 6 \text{ kg ha}^{-1}$  ( $n = 97$ ). This reduction of apparent N loss due to CC use was small in comparison to the total apparent N losses of the control treatments, which amounted to  $217 \pm 17 \text{ kg ha}^{-1}$  ( $n = 42$ ), but significantly different from zero according to a one-sample t-test ( $p = 0.024$ ).

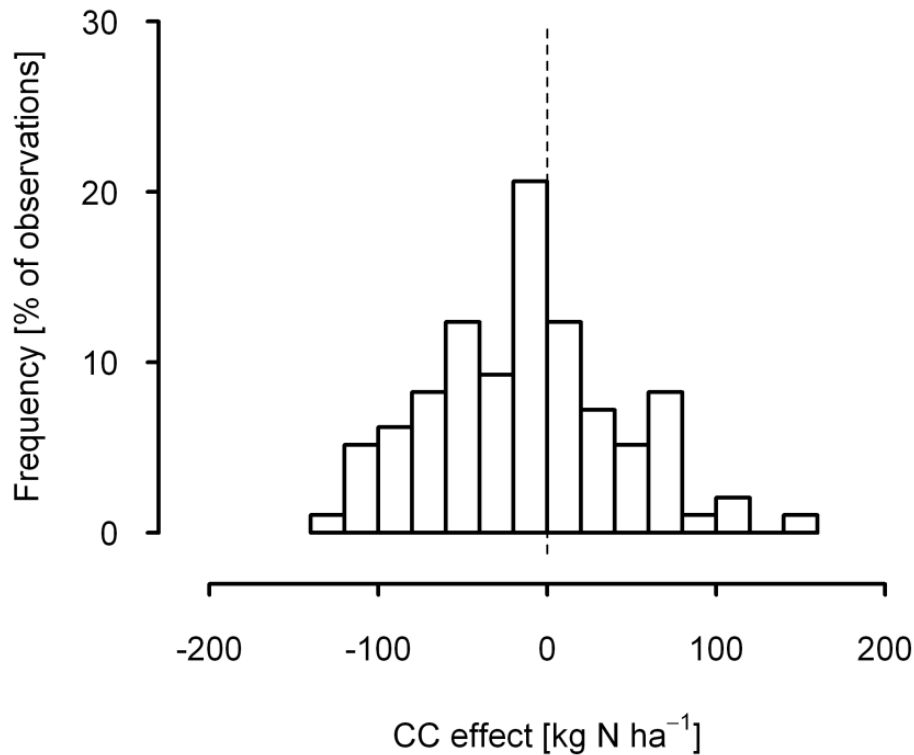


Figure 4-3: Frequency distribution of the CC effect, i.e. the difference in apparent N loss [kg ha<sup>-1</sup>] of the catch crop treatment minus that of the corresponding control treatment. Data comprises all sites, years, and CC treatments ( $n = 97$ ).

The CC effect was significantly affected by the type of CC used ( $p = 0.024$ ), however not by the factor date ( $p = 0.256$ ), according to a factorial ANOVA (Type III SS) which included: type of CC, factor date, their interactions, and a random factor representing the combination of site and experimental year. The corresponding Tukey test indicated that there were significant differences between the sudangrass, which increased the apparent loss by 18 kg N ha<sup>-1</sup> ( $n = 12$ ), and fodder radish treatments, which reduced the apparent loss by 30 kg N ha<sup>-1</sup> ( $n = 34$ ), compared to the control treatments. Moreover, the CC effects were only significant in the bunch onion and fodder radish treatments, whereas for the sudangrass and the winter rye, no significant differences in the apparent N losses of control and CC treatments were found (Fig. 4-4a). Also, when pooling the data according to the factor date, a significant reduction in apparent N losses due to CC use could be found in the early treatments ( $-20 \pm 7$  kg ha<sup>-1</sup>,  $n = 49$ ,  $p = 0.010$ ), whereas no effect was found for the late treatments ( $-6 \pm 9$  kg ha<sup>-1</sup>,  $n = 48$ ,  $p = 0.492$ ).

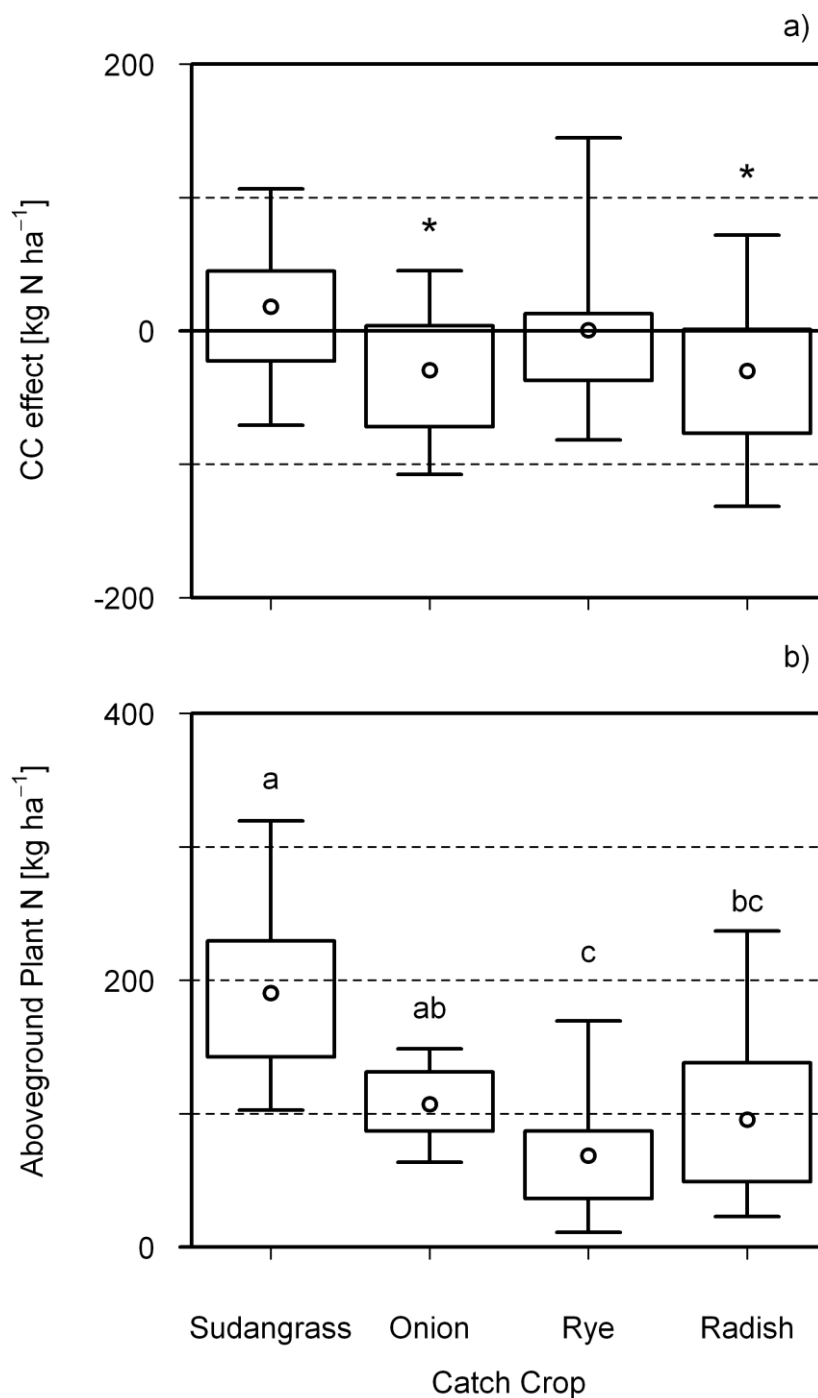


Figure 4-4: Catch Crop effect, i.e. the differences in apparent N loss in kg ha<sup>-1</sup> of the catch crop treatment minus that of the corresponding control treatment (a) and maximum aboveground N contents of catch crops in kg ha<sup>-1</sup> (b), averaged across all experiments. \* Significantly different from zero according to one-sample t-test ( $p < 0.05$ ), catch crops not sharing a common letter (above boxes in 4a) were significantly ( $p < 0.05$ ) different from each other according to a non-parametric post hoc test as suggested by Siegel and Castellan (1988). Sample sizes: sudangrass: 12, onion: 16, rye: 40, radish: 36. Circle: arithmetic mean, hinges: 25% and 75% quantile, whiskers: minimum and maximum values.

When averaged across all experiments, there were significant differences among the maximum aboveground N contents of the different types of CC (Fig. 4-4b). However, these were not significantly correlated to the CC effects ( $r = 0.10$ ,  $n = 97$ ,  $p = 0.335$ ). To assess the N balance surpluses for a system with shallow-rooted succeeding crops, which can only access N in the upper soil sections, the balances were calculated in the same way as previously described, however, only SMN (input and output) in the soil depth sections of 0–30 or 0–60 cm were considered. Reducing the considered soil depth section in this way results in more SMN being “lost” by leaching. It is under such circumstances that a deep-rooted CC has the potential to be particularly effective. However, even in these cases there was no significant correlation between maximum aboveground N contents of the CC and the CC effects (0–60 cm:  $r = 0.03$ ,  $p = 0.748$ ; 0–30 cm:  $r = 0.01$ ,  $p = 0.890$ ;  $n = 97$ ).

### 4.3.3 Relationship between N input variables and apparent N loss

The apparent N loss was significantly correlated with the total N input into the crop rotation ( $r = 0.70$ ,  $n = 139$ ,  $p < 0.001$ ). No such significant linear relationships were observed between the CC effect and total N input ( $r = 0.06$ ,  $n = 97$ ,  $p = 0.580$ ).

## 4.4 Discussion

### 4.4.1 Nitrogen balance and cauliflower crop residues

The average N input across all experiments was a high  $386 \text{ kg ha}^{-1}$ , half of which could be ascribed to the cauliflower crop residues (Fig. 4-2). This demonstrates how important residues from crops like cauliflower and broccoli are for the N balance of whole vegetable crop rotations. These amounts were in the upper range of figures summarized by Feller et al. (2010), who compiled data from many field experiments and reported average values for cauliflower crop residues of between  $153$  and  $180 \text{ kg N ha}^{-1}$ . On average, only half of the total N input was recovered at the end of the crop rotation suggesting that the other half, a high absolute amount of  $203 \text{ kg ha}^{-1}$ , may have been lost.

The results of the N balances calculated using the alternative method stipulated by The German Fertilizer Ordinance, demonstrated that in the presented experiments where the fertilization was based on target values (Feller et al. 2010) and frequent measurements of SMN contents, i.e. fertilizer was applied according to “good agricultural practice”, the

balance surplus limits were met in about half of the investigated cases, an encouraging result. On the other hand, the results indicate that N balance calculations which do not consider SMN contents may greatly deviate from those that include them, and hence, might present a highly biased estimate of actual N losses.

The presented balance calculations may have overestimated apparent N losses if considerable N amounts were immobilized in soil organic matter or were still bound in crop residues at the end of the considered crop rotation period. According to decomposition rate constants obtained by De Neve and Hofman (1996) from fitting monoexponential models to data from incubation experiments, the mineralizable part of total vegetable crop residue N is completely mineralized within one year, even when assuming a soil temperature of only 10°C. The part of total N that was “mineralizable” according to this model varied but generally ranged between 60 and 80% of total N for leafy vegetable residues (De Neve and Hofman 1996). In another study, 80% of the N contained in cauliflower crop residues was recovered in the soil mineral N pool within 3 months after incorporation at temperatures > 16°C (De Neve et al. 1996). The N fraction not recovered within a year can likely be attributed to gaseous N emissions on the one hand and to immobilization in humified soil organic matter or recalcitrant residues on the other hand. Investigations on the humification coefficient of different organic materials indicated that for grass between 12 and 15% of residue C remains in humified organic matter one year after incorporation (Kätterer et al. 2011). Likewise, Henriksen and Breland (1999) performed litter bag experiments and recovered less than 20% of the added C and N two years after incorporation of ryegrass foliage, potato haulm, white clover foliage, and white cabbage leaves.

Hence, it can be assumed that in the present study the turnover of N from cauliflower and CC residues was almost complete after the considered time periods, spanning several months for the CC and more than one year for cauliflower, and that a only a minor fraction of the total residue N was immobilized in soil organic matter. Indeed, it would have been desirable to quantify this immobilized N by means of isotope techniques. However, this was not possible within the framework of this study.

Disregarding possible N outputs (undecomposed crop residue N, humified N) and also N inputs (deposition, mineralization of soil organic matter) may have affected the absolute N balance surpluses estimated in the present study. However, this error was presumably small regarding the key target variable, i.e. the differences in the N balance surpluses between



different catch crop treatments, because the disregarded inputs and outputs were likely not distinctly different among the catch crop treatments at a common site.

#### 4.4.2 Catch crop effects on apparent N loss

Although the apparent N loss of the control treatments without CC was high, the mean CC effect was very low. In some cases, the apparent N losses in the treatments with CCs even exceeded those of the control treatments by  $100 \text{ kg ha}^{-1}$  or more (Fig. 4-3). This high variation in the effect of the CC on the N balance could in part be explained by the type of CC, which had a significant effect on the reduction in apparent N loss according to the ANOVA. Also, the apparent N loss was significantly reduced with fodder radish and bunch onion, whereas there was no reduction for sudangrass or winter rye when compared to the control treatments (Fig. 4-4a). It is difficult to determine which factors led to these differences but apparently maximum aboveground N content of the CC was not the decisive parameter. Sudangrass, which showed the highest N content (Fig. 4-4b), had no significant effect on the N balance. This might be due to the fact that sudangrass was cut and incorporated into the soil already in November of the first year while all other CCs were incorporated in spring of the following year. Thus, some of the N in sudangrass residues may have been mineralized during winter and lost before it could be taken up by the succeeding crop. Prolonged immobilization of N from sudangrass residues was unlikely since their C/N ratios ranged between 19 and 26 (data not shown). Several studies demonstrated that plant residue turnover is maintained at low temperatures even below freezing point and that N mineralization can be significant over winter (e.g. Thorup-Kristensen 1993; Van Schöll et al. 1997). Effects of timing of CC residue incorporation on the N nutrition of succeeding crops have also been observed by Thorup-Kristensen (1993), who reported a positive effect of postponing incorporation from autumn to spring on the N uptake of a carrot (*Daucus carota* L.) crop after a phacelia catch crop (*Phacelia tanacetifolia* Benth.), not however after grass catch crops (*Lolium multiflorum* Lam., *Lolium perenne* L., or *Secale cereale* L. var. *multicaule* METZG. ex ALEF). The authors argued a possible reason could be the higher nitrate content of the phacelia crop compared to grass. However, apart from the aim to reduce N losses, the farmer has to consider many different aspects when choosing the timing of CC residue incorporation. In many field vegetable production systems the priority for the farmers is to plant the next vegetable “cash crop” as soon as possible in spring because it is of paramount importance to their competitiveness.

In addition to leaching losses after CC residue incorporation, we assume that gaseous losses of N occurred which reduced the amount of N available to the succeeding crop. It has been shown that after mulching, but before incorporation of crop residues into the soil, losses of N via ammonia volatilization can reach more than 10% of total applied N in 3–5 weeks (de Ruijter et al. 2010; Glasener and Palm 1995). In the experiments of the current study, the duration between mulching and incorporation of crop residues usually spanned a few days but in some cases extended over weeks. In addition to ammonia volatilization, losses of N via denitrification can be significant after incorporation of crop residues, in particular when both C and N are readily available in the soil. Velthof et al. (2002) reported losses in the form of nitrous oxide of up to 15% of N applied with crop residues in 11 weeks. Schloemer (1991) investigated total gaseous N losses via denitrification after incorporation of cauliflower residues and found that up to 40 kg N ha<sup>-1</sup> was lost in eight weeks. Ammonia volatilization and denitrification losses may have occurred, especially after harvest of cauliflower crop residues but also after the incorporation of CC residues and after frost kill of the fodder radish CC, diminishing the positive effects of the CC on N balance.

In the case of rye, the average maximum aboveground N content was around 60 kg N ha<sup>-1</sup>, the lowest of all CCs. This was a result of the relatively slow development of the rye during the early stages of growth. Bunch onion and fodder radish showed higher aboveground N contents, which were not significantly different from each other. Certainly, part of the positive effect of bunch onion on the N balance (Fig. 4-4a) could be attributed to the harvest of onion, since this removed 88 kg N ha<sup>-1</sup> on average from the system. However, the true value of this strategy compared to other green manuring strategies, in regard to saving more N, depends on the amount of N lost during the processing of these harvested products. Kristensen and Thorup-Kristensen (2004) and Thorup-Kristensen et al. (2003) pointed out that in most cases dicots, especially crucifers (e.g. fodder radish), have a higher potential for N uptake than monocots (e.g. ryegrass, rye) when not sown too late in autumn, due to faster and deeper root development. This finding seemed to be confirmed by the experiments of the current study, at least with respect to bunch onion, rye, and fodder radish. Also, Kristensen and Thorup-Kristensen (2004) argued that aboveground plant N content alone is not a good indicator of the efficiency of a CC, since differences in the root to shoot ratio with respect to N, as well as N deposited with dead plant matter during CC growth, are not considered. This may have contributed to the absence of a correlation between aboveground plant N content and CC effects in this study. Also, it is difficult to compare different CC species when other factors,

like harvest date and method and timing of crop residue incorporation, differ. It has to be taken into account that, with exception of bunch onion, the rooting depth of the CCs used in the current experiments may have been considerably deeper than the 90 cm considered in the balance calculations. This might have contributed to lower apparent N losses in some CC treatments due to translocation of N from deeper soil layers to the topsoil. Kristensen and Thorup-Kristensen (2004) and Thorup-Kristensen (2001) suggested that the differences in CC efficiency can be mainly determined by differences in SMN uptake by the CC from soil depths below 1 m.

#### **4.4.3 Other factors affecting the N balance**

When pooling the data according to the factor date, only the early treatments significantly reduced the apparent N loss. This was in line with expectations since a CC that has more time to develop in autumn can take up more N and hence prevent N losses better than a poorly developed crop with an insufficient root system. However, the difference in reduction of apparent N losses between the early and the late treatments of only 14 kg N ha<sup>-1</sup> could be considered irrelevant to farming practice.

As expected, the apparent N losses were significantly correlated with total N input, demonstrating that when more N is put in the system, more N is lost. Notably, the effect of the CC, estimated by the reduction in apparent N loss by a CC treatment compared to the corresponding control treatment, was not significantly correlated with total N input. The effect of CC was expected to increase with N input since in systems with high potential for N losses CCs can substantially decrease N losses, whereas in systems that do not exhibit N losses in the first place, the effects of CC should be negligible or even negative. The latter can occur when N that was taken up by the CC would otherwise, without a CC, be retained in the soil, an effect which Thorup-Kristensen (1993) termed pre-emptive competition. In the current experiments, the effects of the CCs were apparently too small, in relation to the unsystematic variation by random error, for this expected relationship between N input and CC effect to be observed. This was in part a result of the great variability from year to year, mainly imposed by variable weather conditions, which made the detection of average CC effects difficult. Rankinen et al. (2007) suggested that N balances should be calculated for periods longer than one year in order to reduce the huge effects of seasonal precipitation and cultivation practice. The same applies to the presented experiments on whole crop rotations spanning two years, since weather conditions during the winter period, which is at high risk of leaching losses, have a great and variable impact on CC effects and on N balance (e.g. Beaudoin et al. 2005).

This problem can only be overcome by performing experiments in time series at a certain site and hence increase the statistical power in separating systematic CC effects from the effects of random factors. However, this is very laborious and costly and will only result in recommendations specific to the investigated site. Another promising option is the use of agro-ecosystem models like Daisy (Abrahamsen and Hansen 2000) to predict the C and N dynamics of whole vegetable crop rotations, simulate the N balances, and use the results as a criterion for the effectiveness of different CC strategies to reduce N losses.

#### **4.4.4 Conclusions**

The typical intensive German vegetable crop rotations investigated in this study were particularly prone to N losses due to the high input of mineral N fertilizers, as well as the high amount of SMN and crop residue N left after harvest of the cauliflower crop. However, based on the large data set obtained from performing field experiments, at three different sites, over 2–3 years, and after applying several CC treatments, we conclude that the use of winter CCs, in combination with conventional mineral N fertilization does, for many sites, not solve the problem of high N balance surpluses in these systems. This was evidenced by the small effects of the CCs on the N balance surpluses in comparison with the much higher total N balance surpluses, as well as from the high uncertainty associated with these CC effects. For these sites, alternative measures, such as the harvest of non-food vegetable parts, vegetable-cereal rotations, or precision farming may be more effective in reducing N losses.

#### **Acknowledgements**

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## **5 General discussion and conclusions**

### **5.1 Fertilization history effects on decomposition**

In the soil, organic matter is continuously degraded, i.e. it is gradually converted into other compounds. Consequently, some organic matter is lost by mineralization to inorganic compounds, e.g. as CO<sub>2</sub> gas or NH<sub>4</sub><sup>+</sup> in solution. However, the components of organic matter show different resistance to degradation, and thus the chemical structure of the remaining fraction of the organic matter changes during decomposition (Heal et al. 1996). The latter conversion in the end leads to the formation of complex organic macromolecules, which constitute the soil humus, a process called humification (Stevenson 1994). In view of this complex process of decomposition, it becomes clear that only one measure, like CO<sub>2</sub> evolution for instance, can not reflect the whole process entirely. Hence, whether fertilization history has an effect on the “decomposition” of recently added organic fertilizer may depend on the investigated variable chosen to represent decomposition.

The investigated variables typically depend on the research question but also on the available techniques. Net N mineralization from applied organic fertilizer is certainly the measure that is most relevant to fertilization practice. However, from a scientific point of view, net changes in mineral N contents can be regarded as “symptoms” of many different processes that actually control net mineral N contents, such as: gross N mineralization, gross N immobilization, plant N uptake, fixation of mineral N during humification (Jansson and Persson 1982). It may occur that several processes are affected by an experimental treatment but no change in net N mineralization is caused due to the compensation of counteracting processes. It should be the task of fundamental research to disclose the underlying mechanisms of effects, irrespective of their practical relevance and degree of observability.

In this study, it was attempted to cover both describe effects of fertilization history on net N mineralization and analyze some of the potentially responsible processes.

### 5.1.1 Effects on net N mineralization

The pot experiment presented in Chapter 2 spanned a period of 10 weeks, at the end of which between 2.0 and 4.5% of the added organic N in farmyard manure ( $570 \text{ kg N ha}^{-1}$ ), depending on fertilization history, was mineralized (Fig. 2-1). This fertilization history effect on net N mineralization from farmyard manure was high in terms of relative measures but corresponded to an absolute amount of only  $14 \text{ kg N ha}^{-1}$ . This slow net N mineralization of composted farmyard manure was consistent with figures reported by Hadas et al. (1996) and can be explained by stabilization processes of organic N as well as a reduction in  $\text{NH}_4^+$  content during composting (Eghball et al. 1997). The findings of the incubation experiment (Chapter 3) confirmed the slow net N mineralization of farmyard manure and illustrated that this was not a consequence of prolonged microbial N immobilization but of a slow turnover (Fig. 3-2, bottom row).

The incubation experiment presented in Chapter 3 lasted 21 weeks and hence much longer than the pot experiment. Also, during the incubation soil moisture and temperature were kept within the optimal range for decomposition. Nevertheless, the effects of fertilization history on net N mineralization were absent at the end of the incubation as there was no significant interaction between fertilization history and recent fertilization for soil mineral N contents. The results suggested that the reasons for the absence of effects of fertilization history on net N mineralization depended on the type of fertilizer. A general relationship may be hypothesized, described as follows. For materials that are rapidly decomposed and also contain a substantial amount of N, the effects of fertilization history on turnover and thus net N mineralization are negligible. This was the case in fresh cabbage residues applied in the incubation experiment, which contained a total N amount of  $214 \text{ kg ha}^{-1}$  and were completely mineralized within 10 weeks. For materials that are more recalcitrant, the relative differences in turnover rates caused by different fertilization histories may be more pronounced but the resulting absolute differences in net N mineralization are of minor relevance due to the slow turnover rates and/or small total N contents.

Relationships between chemical characteristics of organic materials and their C and N mineralization, such as those described by Trinsoutrot et al. (2000b), Thuries et al. (2002), and Jensen et al. (2005), may serve as a tool to distinguish between both kinds of materials. The rapid mineralization of the cabbage material in the incubation experiment was in accordance with data reported by Jensen et al. (2005), who incubated 76 plant materials and generally found more than 50% of the added C mineralized within 10 weeks for materials with a holocellulose content of less than  $150 \text{ mg C g}^{-1}$  dry matter. Henriksen and Breland (1999)

reported a holocellulose content of white cabbage leaves of  $77 \text{ mg C g}^{-1}$  dry matter. In contrast, pine bark exhibited a slow turnover compared to cabbage material, which could be attributed to the very high holocellulose and lignin content of pine bark (Valentín et al. 2010). In addition, the applied pine bark contained only  $49 \text{ kg N ha}^{-1}$ . Both, slow turnover and small N content, made the detection of effects of different turnover rates on soil mineral N contents unlikely, although fertilization history effects were more pronounced than in the other recent fertilization treatments. In case of farmyard manure, slow turnover rates and minor differences in turnover rates between fertilization histories led to the absence of fertilization history effects on soil mineral N contents, although the manure contained a high amount of  $500 \text{ kg N ha}^{-1}$ .

### 5.1.2 Effects on gross C and N turnover

Calculating the N balances in the pot experiment (Chapter 2) by using soil mineral N, microbial biomass, and plant N measurements provided rough estimate of gross N turnover of the recently added farmyard manure. The results indicated that there were no differences in this gross N turnover among the different organic fertilization histories after 10 weeks ( $\Delta N_{\text{FYM}}$ , Fig. 2-1). In the balance calculations however, it is implicitly assumed that all N that is fixed in microbial biomass becomes immediately available as soil mineral N upon microbial death. This does not reflect reality, i.e. that only a part of the microbial N is directly released as mineral N upon microbial death, another part is left in microbial residues and some N is incorporated into soil organic matter (Shen et al. 1984, Grant et al. 1993). These processes were all included in the model used to analyze the data from the incubation experiment (Fig. 3-1). Using this model, it was possible to obtain an estimate of gross decomposition, taking into account the observations of  $\text{CO}_2$  evolution, soil mineral N contents and soil microbial biomass contents. This approach can hence be regarded as superior to the approach used in the balance calculations of the pot experiment. Furthermore, the estimation of the model parameters that reflect the turnover of previously existing soil organic matter was based on a data set of four recent fertilization treatments. The resulting estimate of the “background” mineralization can be considered more reliable than the estimate based on only one control treatment, as it was the case in the pot experiment.

Using the modeling approach, the simulated amounts of added organic matter C that were decomposed by the end of the incubation period differed among the fertilization history treatments by up to 6% of the added C for farmyard manure and up to 21% for pine bark (Fig. 3-2). There were no differences for cabbage recently added as this was completely

decomposed 75 days after application in all soils. Moreover, the courses of the decomposition of the cabbage material were very similar among the fertilization history treatments (Fig. 3-2). This was also confirmed by the results of the model fitness indices (*AICc*, Table 3-3), which indicated that fertilization history should be considered when modeling the decomposition of farmyard manure and pine bark, not however when modeling the decomposition of cabbage.

In the model, organic matter turnover was modeled on the basis of C dynamics and, based on C/N ratios that are specific for each organic matter pool and constant in time, the N dynamics are simulated accordingly. The corresponding absolute amounts of N that disappeared from the added organic matter pool can be regarded as gross N mineralization, i.e. parts of it were mineralized to soil mineral N and parts of it were incorporated into microbial biomass or soil organic matter. The greatest differences among the fertilization history treatments in simulated gross N mineralization from recently added organic matter at the end of the incubation corresponded to absolute amounts of 38 kg ha<sup>-1</sup> for farmyard manure and 13 kg ha<sup>-1</sup> for pine bark (data not presented in Chapter 3).

In view of these modeling results, it can be concluded that there was some evidence for fertilization history effects on the decomposition of organic fertilizers that are more recalcitrant than vegetable crop residues. However, these effects did not exhibit a consistent increase or decrease in gross C and N mineralization due to long-term fertilization history. It needs to be kept in mind that a simulation model can only represent very simplified reflection of the processes that exist in reality. In particular the flexibility of the microbial biomass in the soil with its dynamic metabolic activity, nutrient contents, and community structure can only marginally be mirrored by the used model, which comprised two microbial biomass pools with turnover rates, C use efficiencies, and C/N ratios that were constant in time. It would thus be interesting to investigate the observed decomposition patterns with a model that features a more complex description of microbial dynamics, such as the model proposed by Blagodatsky and Richter (1998).

The fact that organic matter such as cabbage material, which, compared to farmyard manure and pine bark, is very easy to decompose, exhibited the same turnover rate in all soils is supported by some results from biodiversity research (see next section).



### **5.1.3 Biodiversity and basic ecosystem functions**

It has been shown that the biodiversity of organisms involved in the decomposition process is altered by long-term organic fertilization practice (e.g. Buckley and Schmidt 2001, Ruppel et al. 2007, Stark et al. 2008). Does this change in biodiversity have an impact on the decomposition of newly added organic material?

There is convincing evidence that a higher biodiversity reduces the variability in e.g. microbial biomass (Naeem and Li 1997) and respiration (McGrady-Steed et al. 1997) of undisturbed systems as well as increases the system's resistance and resilience to perturbations (Loreau et al. 2001). The authors attributed this to the redundancy in basic ecosystem functions, i.e. the fact that these functions can be fulfilled by various species alike. This redundancy also suggests that fertilization history effects on decomposition of materials that represent a common substrate for soil organisms, such as crop residues, can be neglected, at least by means of an altered biodiversity. This theory is supported by Andrén et al. (1995) and Griffiths et al. (2001), who found no consistent effects of artificially reduced biodiversity on the decomposition of barley straw. On the other hand, it has been shown that even for several bacterial or fungal species, all of which are capable of degrading cellulose on its own, a synergistic effect with respect to decomposition can be observed in coexistence, presumably due to small functional dissimilarities (Hättenschwiler et al. 2011). A review paper by Nielsen et al. (2011) indicated that among the studies investigating biodiversity effects on C decomposition, the ones involving systems with less than 10 species much more often showed positive effects of increased biodiversity on decomposition than studies involving more species. The authors argued that a decrease in biodiversity of naturally species-rich soil communities is unlikely to reduce decomposition unlike species with very special functions are eliminated. Hence, results from biodiversity research suggest that for materials that are easy to decompose and thus can be utilized by most organisms in the soil, reduced microbial biodiversity or altered microbial community structure should be of minor importance, at least as long as biodiversity does not fall below a critical level. This is in line with the results of the current study, viz. that effects of fertilization history on the decomposition of recently added organic fertilizers were absent for cabbage material, which as leafy plant litter is common to the organisms in arable soil, while effects were more pronounced for farmyard manure and pine bark, which can be considered alien substrates to these organisms.

### 5.1.4 Technical remarks – considered processes and applied methods

A potential source of error in decomposition studies that do not involve isotope techniques is the occurrence of priming effects. Priming effects are effects of the experimentally applied treatment on processes not originally targeted in the experiment, which in turn affect the observed variables of interest. In the current context, recent application of organic fertilization can change the turnover of previously existing soil organic matter, by means of altered soil microbial activity, and thus cause a net change in, e.g. soil mineral N contents (Kuzyakov et al. 2000). In addition, positive feedback effects may occur, i.e. the process that is unintentionally triggered causes a change in the investigated variable that further up-regulates this process. This can for instance be the case when N mineralization causes a rise in plant growth, which in turn increases rhizodeposition. This rhizodeposition further stimulates microbial activity and hence N mineralization (Paterson 2003). The same applies in the opposite direction, i.e. a process that is unintentionally reduced indirectly down-regulates itself.

The neglect of the described priming and feedback effects often leads to misinterpretation of experimental results. In the experiments presented in the current thesis, priming effects were not accounted for, since the determination of these effects is very difficult, basically only feasible by means of isotope enrichment techniques. In particular priming effects mediated by the fodder radish plants in the pot experiment of Chapter 2 could therefore not be ruled out.

Future research on this topic, i.e. the investigation of potential processes responsible for fertilization history effects on the decomposition of recently applied organic matter, could benefit from combining following methods:

- full factorial design of historic and recent fertilization treatments
- isotope labeling of the investigated nutrients (e.g.  $^{13}\text{C}$  or  $^{15}\text{N}$ ) in recently applied fertilizer to be able to trace it and separate it from other sources
- mechanistic modeling, in combination with measurements of nutrient contents in different soil compartments (e.g. microbial biomass, soil mineral N, gaseous emissions)
- monitoring of changes in the microbial community structure during the experiment

It could be worthwhile to attempt the separation of microbial communities from the soil they inhabit to enable the segregation of effects mediated by the microbial community, effects derived from differences in abiotic soil properties, and interactions of these effects.

### **5.1.5 Conclusion**

The hypothesis that fertilization history effects on the decomposition of recently applied fertilizers occur for recalcitrant fertilizers, not however for easily decomposable fertilizers, was accepted. The results supported the hypothesis in that the turnover rates estimated in the incubation study showed the greatest differences between fertilization histories for pine bark, which was the most recalcitrant of the applied fertilizers, and the least differences for cabbage, which was the most easily decomposable fertilizer. Altogether, however, the results of both studies showed no consistent trend with respect to fertilization history effects on C and N mineralization. If present, effects seemed to be minor in importance for net N mineralization and hence fertilization practice. In the objectives of this study it was stated that to increase the N use efficiency in agricultural systems, one measure could be to improve the prediction of N availability to the crop after organic fertilization. This statement is principally true but it seems that the consideration of organic fertilization history does not substantially improve the prediction of net N mineralization from organic fertilizers.

Although it may not be relevant to fertilization practice, the understanding of processes that drive the changes in microbial decomposing activity as a result of long-term fertilization might prove a valuable knowledge for predicting consequences of human actions on this key function of soils and hence for conserving soil fertility.

## 5.2 Catch crop strategies to reduce N losses

### 5.2.1 Effectiveness of catch crops in reducing N balance surpluses and alternative measures

The results of the current study (Chapter 4) demonstrated that the inclusion of winter catch crops (CC) in the investigated intensive vegetable crop rotations reduced the N balance surpluses on average by less than 30 kg N ha<sup>-1</sup> (Fig. 4-4a). In fact, CC do not always reduce N balance surpluses, in some cases, the surpluses may even increase with CC cultivation (Fig. 4-3). This was a remarkable finding since results from many other studies showed significant and large reductions in N leaching by cultivation of winter CC compared with the fallow control (e.g. Wyland et al. 1996, Beaudoin et al. 2005, Askegaard and Eriksen 2008). However, some studies support the results of the current study. For instance, Martinez and Guiraud (1990) indicated that a ryegrass CC reduced the N uptake of a succeeding maize crop in a lysimeter study, which they attributed to prolonged immobilization. Burket et al. (1997) reported that the recommended fertilization N rate in mixed vegetable crop rotations with fallow over winter could not be reduced without yield loss of the principal crop when a rye winter CC was used. Also, Herrera and Liedgens (2009) found that winter CC effectively reduced N leaching over winter but did not increase the N use efficiency when considering the entire crop rotation. These results highlight the investigated time scale as a factor of capital importance to the evaluation of management options on the N balance. At least whole crop rotations should be considered, to overcome the high temporal variability induced by seasonal precipitation and management events (Rankinen et al. 2007) and to account for long-term residual effects of CC cultivation (Hauggaard-Nielsen et al. 2009).

The current study further indicated that the effect of CC on the N balance cannot be simply derived from the maximum N content of the CC. This was because a complex interplay of several factors determine the trade-off between pre-emptive competition for N between CC and succeeding crop and the prevention of N losses by CC uptake, as was also demonstrated by Thorup-Kristensen and Nielsen 1998. The success of CC cultivation in reducing the N balance of the whole crop rotation thereby depends on two critical steps, the N uptake of the CC and the mineralization of CC residues. It seems that the transfer of CC residue N to the succeeding crop remains the least predictable part of the entire process. Apart from the well-known relationships between decomposition and temperature and moisture (Cabrera et al. 2005) as well as the chemical composition of the residues (Heal et al. 1996), prolonged N immobilization in organic forms and gaseous losses of N may have been underestimated in

many studies. This has probably been in part responsible for typical recovery rates of CC residue N between 0 and 50% (Thorup-Kristensen et al. 2003). The accurate recovery of CC residue N could not be calculated in the current study since no isotope techniques were used. However, comparing the maximum N contents of the CC, which were higher than 50 kg N ha<sup>-1</sup> for all types of CC (Fig. 4-4b), to the average effects of these CC on the N balances, which were always less than 50 kg N ha<sup>-1</sup> (Fig. 4-4a), suggests that the greatest potential for improvement lies in the lossless transfer of CC residue N to the succeeding crop.

Hence, to include an additional CC in the crop rotation adds high uncertainties to the N balance of the whole crop rotation. To avoid part of these uncertainties and yet have an alternative to bare soil over winter, the removal of CC biomass in spring may be a useful tool. The results of the N balances suggested that bunch onion as a CC reduced the N balance surplus due to removal of the harvest. At the same time, a marketable product was harvested. The true value of this measure will be determined by the post-harvest losses of reactive N.

From the results of the current study it can be concluded that even when the N uptake capacity of the system is increased by adjusting the crop rotation, the amount of N temporarily stored in biomass is still at risk of being lost. Hence, it seems that of the management options mentioned in the objective of this study (section 1.5), the adjustment of amount and type of the N input (point 1) could be most rewarding. The reason is that once reactive N has been put in the system it is always at risk of being lost unlike retained fertilizer N. In this context, precision support tools for more accurate spatial distribution of fertilization and irrigation on the field may be of key relevance (Robertson and Vitousek 2009).

### **5.2.2 Technical remarks – the inevitable variation in time and space**

Nitrogen dynamics are subject to high temporal and spatial variability due mainly to the impact of the weather and the natural heterogeneity of soil properties, respectively. When performing field experiments, this variation makes the detection of systematic effects imposed by the experimental treatments very difficult. One solution could be to set up experiments with a high number of replications both in time and space for every desired combination of soil, weather, and management conditions. However, this is very costly and laborious. Therefore, in this study it was originally planned to calibrate and validate an agroecosystem model using the data from the performed field experiments. The aim was, (i) to demonstrate that the model produces reasonable results, and then (ii) to run scenario calculations to identify crop rotations that are suitable of reducing N losses at different sites and under

different weather conditions. Unfortunately, none of the tested models, EU-Rotate-N (Rahn et al. 2010) and DAISY (Abrahamsen und Hansen 2000), produced results that were accurate and reliable enough to permit the planned scenario calculations (results not presented). Therefore, the obtained data from the field experiments were analyzed by means of basic statistics and some shortages of the experimental design had to be accepted.

The N balance surpluses as calculated in the current study could be used to assess the suitability of the chosen crop rotation strategy to increase the N uses efficiency of these systems. Yet, the components of the N balances, such as plant N and soil mineral N contents, could only suggest possible causes of discovered differences in the N balances. Identifying N flows by separating different N sources, such as catch crop residue N and previously existing soil organic matter N, could give a deeper insight in the processes that occurred. For future research, it seems also particularly challenging to determine the proportions of different pathways of N losses to the total N balance deficit in these systems. Gaseous N losses, for instance, have received high attention in recent years due to the effect of some nitrogenous gases on the global climate. The quantification of total gaseous N losses and the contribution of different N gases after incorporation of vegetable crop residues remains a challenging task and could be targeted in future research.

### **5.2.3 Conclusion**

The hypothesis that catch crops can substantially reduce N losses in German intensive vegetable production systems could not be confirmed by the results of this study. The investigated systems were especially prone to N losses due to the high input of mineral N fertilizer and the cauliflower crop at start of the crop rotation. In such systems, it could be rewarding to draw the attention also to alternative measures that have the potential to mitigate N losses, such as precision farming, removal or treatment of crop residues, or the timing, placing, and formulation of N fertilizers. The solution to high N balance surpluses in agricultural systems and associated environmental damage will not be solved by a single new technology or practice. As Robertson and Vitousek (2009) claimed so accurately, a portfolio approach is required, i.e. the combination of technological progress, social motivation, and the ongoing evaluation of the success of measures that have been taken.

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